ANNUAL REVIEW OF MICROBIOLOGY

CHARLES E. CLIFTON, *Editor*
Stanford University

SIDNEY RAFFEL, *Associate Editor*
Stanford University

ROGER Y. STANIER, *Associate Editor*
University of California

VOLUME 8

589.95
An7
v.8

1954

ANNUAL REVIEWS, INC.

STANFORD, CALIFORNIA, U.S.A.

ANNUAL REVIEWS, INC.
STANFORD, CALIFORNIA, U.S.A.

FOREIGN AGENCIES

H. K. Lewis & Company, Limited
136 Gower Street
London, W.C. 1

Maruzen Company, Limited
6, Tori-Nichome Nihonbashi
Tokyo

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA BY
GEORGE BANTA PUBLISHING COMPANY

PREFACE

These prefatory paragraphs provide another opportunity for us to express our thanks to those who have collaborated in the preparation of this *Review*. We are particularly indebted to the contributors for the time and care they have given to the writing of these reviews. In the broad and expanding field of microbiology the magnitude of the material to be reviewed in the space that is available makes their endeavors difficult. The tremendous growth in interest in certain of the fundamental fields is a healthy sign for the future of microbiology.

We wish to extend our thanks to Dr. W. H. Taliaferro who is retiring this year from the Editorial Committee, and to welcome Dr. T. M. Sonneborn as a new member. Dr. Taliaferro has graciously contributed much time and effort in the founding and development of this *Review*. It is a pleasure to announce the appointment of Dr. R. Y. Stanier as an associate editor.

We extend once again our appreciation for the valuable assistance and cooperation extended by the office staff of Annual Reviews and for the care exercised by our printer, the George Banta Publishing Company.

M.D.E.	P.W.W.
E.C.S.	S.R.
J.M.S.	R.Y.S.
T.M.S.	C.E.C.

TOPICS AND AUTHORS
ANNUAL REVIEW OF MICROBIOLOGY
Volume 9 (1955)

TAXONOMY, *R. E. Buchanan*
MORPHOLOGY OF VIRUSES, *F. B. Bang*
GENETICS OF MICROORGANISMS, *M. R. Zelle*
GROWTH OF BACTERIA, *A. Novick*
NUTRITION OF MICROORGANISMS, *E. L. R. Stokstad, H. P. Broquist, and N. H. Sloane*
METABOLISM OF MICROORGANISMS, *E. A. Delwiche*
ANTIBIOTICS, *H. Eagle and A. K. Saz*
NEISSERIA AND NEISSERIAL INFECTIONS, *H. W. Scherp*
PSITTACOSIS-LYMPHOGRANULOMA VENEREUM GROUP, *E. Weiss*
PLAGUE, *G. Girard*
COXSACKIE GROUP OF VIRUSES, *G. Dalldorf*
NEUROTROPIC VIRUSES, *S. Gard*
FUNGUS INFECTIONS OF PLANTS, *C. J. Eide and C. M. Christensen*
PROBLEMS OF INCUBATION IN PLANT DISEASES, *H. Kern*
EFFECT OF IONIZING RADIATION ON INFECTION AND RESISTANCE, *D. W. Talmage*
IMMUNIZATION, *G. Edsall*
IMMUNOCHEMISTRY, *J. R. Marrack*
BACTERIAL SPORES (WITH REFERENCE TO THEIR RESISTANCE AND TO GERMINATION AND ITS INHIBITION), *C. F. Schmidt*

CONTENTS

Cytology of Bacteria

(Part I: The Bacterial Cell), *S. Mudd* 1

(Part II: The Bacterial Nucleus), *E. D. DeLamater* 23

The Nutrition of Microorganisms, *D. Hendlin* 47

Metabolism of Microorganisms, *J. J. R. Campbell* 71

The Chemoautotrophic and Photosynthetic Bacteria, *C. B. van Niel* 105

Leptospira, *H. Schlossberger and H. Brandis* 133

Life History of Malaria Parasites, *P. C. C. Garnham* 153

Mechanisms of Antibacterial Action, *W. W. Umbreit* 167

Genetics of Microorganisms, *S. Spiegelman and O. E. Landman* . 181

Bacterial Virus (with Particular Reference to Synthesis of), *E. A. Evans, Jr.* 237

Changes in the Host Flora Induced by Chemotherapeutic Agents, *E. McCoy* 257

Physiological Aspects of Protozoan Infection, *B. G. Maegraith* . 273

Hepatitis, Yellow Fever, and Dengue, *W. P. Havens, Jr.* . . . 289

Antigenic Variation of Influenza Viruses, *M. R. Hilleman* . . 311

Labelled Antigens and Antibodies, *A. H. Coons* 333

Immunological Properties of Polysaccharides from Animal Parasites, *J. Oliver-González* 353

Nonspecific Factors in Immunity, *W. J. Nungester* 363

Effects of Viruses on Tumors, *A. E. Moore* 393

Bacterial Toxins, *C. L. Oakley* 411

The Microbiology of Dairy Products, *J. J. Jezeski* 429

Foods and Feeds from Fungi, *F. S. Thatcher* 449

Cytopathology of Virus Infections (Particular Reference to Tissue Culture Studies), *J. F. Enders* 473

Indexes 503

Annual Reviews, Inc., and the Editors of its publications assume no responsibility for the statements expressed by the contributors to this *Review*.

CYTOLOGY OF BACTERIA[1,2]
PART I: THE BACTERIAL CELL[3]

By Stuart Mudd[4]

Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Complex metabolic activities are conducted with utmost nicety and precision within the minute cells of bacteria, and the over-all patterns of energy yielding and of synthetic reactions strikingly resemble the corresponding metabolic patterns of the cells of higher organisms. Moreover, genetic continuity and phenomena of genetic change in bacteria exhibit elements of remarkable similarity to, as well as of difference from higher forms. There seems to the present reviewers, therefore, little occasion for surprise in the fact that cytological studies of the most recent years are revealing basic similarities in the structural organization of bacterial cells to those of higher forms (1). The recognition of centers of coordinated enzymatic oxidation-reduction reactions (mitochondria) in the cytoplasm (2), and of nuclei exhibiting on miniature scale the familiar configurations of mitosis (3, 4), are cases in point, although it must be remarked that neither of these instances has yet passed the stage of controversy. The present review strives to present the morphological organization of the normal bacterial cell as the present reviewers, in light of currrent knowledge, believe it to be. Recent literature considered to be relevant is reviewed in so far as is practicable; the impossible task of including all recent works on bacterial cytology is not attempted. To those to whom the picture, sketched within obvious limitations seems inadequate, we offer our apologies, together with a quotation from the *Philosophical Magazine* of one hundred and thirty-six years ago (5):

> Where a conclusion appears simple, the careless observer is apt to suppose it easily attained. Yet though sound philosophy tends always to simplification, the rare quality of simplicity is scarcely ever the flash of intuition, but the slow fruit of close and patient investigation.

Bacteria share with other cells the essential features of biological organization: cell walls and septa affording protection and contributing to defining shape and size; a protoplast, with the potentiality of reversible sol-

[1] The survey of literature pertaining to this review was completed in February, 1954.

[2] The following abbreviations have been used in this chapter: RNA (ribonucleic acid); DNA (desoxyribonucleic acid); PNA (pentosenucleic acid).

[3] This work has been aided by a grant from the United States Atomic Energy Commission, AEC Contract No. AT(30-1)-1342.

[4] It is a pleasure to acknowledge the assistance of Dr. Philip E. Hartman in assembling the material for the present review.

gel transformation, enclosed in a cytoplasmic membrane which regulates exchange with the external medium; within the protoplast a true nucleus and centers of coordinated, enzymatic oxidative-reductive processes, the functional equivalents of mitochondria; within the protoplast also stored inclusions of fat, carbohydrate, and high-energy phosphate, and a highly refined organization of function whose structural basis still can be imagined only vaguely; organs of locomotion, the cilia, and often extracellular capsules or amorphous slime. Various bacteria are known to possess highly-specialized metabolic capabilities, such as the ability to oxidize iron or sulfur, to fix atmospheric nitrogen, to grow at high temperatures, or, in the case of pathogens, the possession of a whole armory of defensive and offensive characters adapting them to parasitism. Genetic continuity, we believe, has been demonstrated in certain well-studied bacteria to be maintained by mitotic nuclear division, probably complemented by accessory features such as reversible, polyploidization (3, 6, 7). Mechanisms of genetic change are in a very early stage of discovery but may be seen already to include meiosis and such special mechanisms as transformation and transduction (8, 9). The methods by which such metabolic and genetic attributes may be discovered and explored have attained a refinement not yet available for investigation of the structural substrata which must underlie the manifold functions. Further refinements of preparative procedures and of applications of light and electron optical equipment will doubtless serve both to consolidate recognition of the basic community of micro- and macrobiologic forms and to disclose specific differentiations of structure implementing differentiated functions.

Extracellular Capsules

Extracellular capsules containing type-specific polysaccharides have been demonstrated as envelopes outside the cell walls of pneumococci, *Klebsiella*, *Neisseria*, *Hemophilus*, *Torula histolytica*, etc. [reviewed by Taylor (10), Duguid (11), Tomcsik (12)]. A nonantigenic extracellular hyaluronic acid material is demonstrable about the cells of mucoid strains of *Streptococcus pyogenes*. Most recently clear-cut morphological differentiation has been demonstrated in the extracellular capsules of species of the genus *Bacillus* by Tomcsik and Guex-Holzer. The capsules in some members of this genus comprise a structural framework continuous with the cell wall, the interstices of which are filled by a second chemical component; the components are recognized to be a group-specific polypeptide composed of D-glutamic acid and one or more type specific carbohydrate polymers.

The polypeptide and polysaccharide components of the capsules of certain species of *Bacillus* have been isolated separately by Tomcsik (13) and Tomcsik & Guex-Holzer (14) and specific immune serum has been prepared against each component. Following reaction with specific antipolysaccharide serum the capsules show dense transverse septa, which are continuous with the cross walls separating the individual bacteria within the chain, and which extend outward to the surface of the capsule; between and parallel to

the dense transverse septa fine striations are visible in the capsule (Plate I, Fig. 1). Treatment with specific antipolypeptide serum demonstrates the polypeptide component within the polysaccharide frame-work (Plate I, Fig. 2). By combining specific antipolysaccharide serum and lysozyme, Tomcsik & Guex-Holzer (15) have observed the dissolution of the capsular structures step by step. Incidental observations are that the bacillary cell wall after dissolution of the capsule reacts with the antipolysaccharide but not with the antipolypeptide serum; also that the end result of the action of the mucopolysaccharide-dissolving lysozyme is the liberation from the bacillary cells of the rounded protoplasts. Tomcsik & Guex-Holzer interpret their observations as indicating the framework of the capsules to be polysaccharide, in the interstices of which the polypeptide is held, an interpretation which is stongly supported by immunochemical data with specific absorbed and unabsorbed antipolysaccharide and antipolypeptide sera (16, 17). Tomcsik & Guex-Holzer (18, 19) have elaborated a new principle for demonstration of capsules. The capsulated organism is reacted with a protein at a pH intermediate between the isoelectric point of the capsular components and that of the protein. The nonspecific interaction renders the capsule visible under the phase-contrast microscope.

Iványovics (20) and Iványovics & Horváth (21) also have demonstrated capsules and capsular components outside the cell walls of *Bacillus sp.* They interpret the capsular framework as D-glutamic acid polymer and the granular material in the interstices as polysaccharide, a view which, in the reviewer's judgment, lacks adequate immunochemical support.

The significance of capsules, surface films, and components of the cell wall in the host-parasite relationships of virulent microorganisms is reviewed by Mudd (22, 23). In general, invasive forms have acquired surface components which protect them from the defensive mechanisms of the host, in particular from destruction by phagocytes.

Duguid (11) reviewed experimentally various methods which have been described for demonstrating bacterial capsules and slime. "The wet-film India-ink method was preferred to all others, as the most reliable, informative and generally applicable." Duguid & Wilkinson (24) have studied quantitatively intracellular polysaccharide content as measured by periodate-Schiff staining, capsular polysaccharide as measured by capsule diameter, and loose slime polysaccharide as measured chemically in the supernatant fluid, in relation to conditions of culture. A dye, Alcian Blue 8GN150, which stains bacterial capsular polysaccharides without previous mordanting, is described by Novelli (25) and by McKinney (26). Gerstley & Morton (27) recommend Fontana's procedure. Sterne (28) describes a Muir's stain modified by addition of a surface-active agent.

Flagella

A comprehensive critique on bacterial flagella, with electron micrographs and extensive bibliography, has been published recently by van Iterson (29).

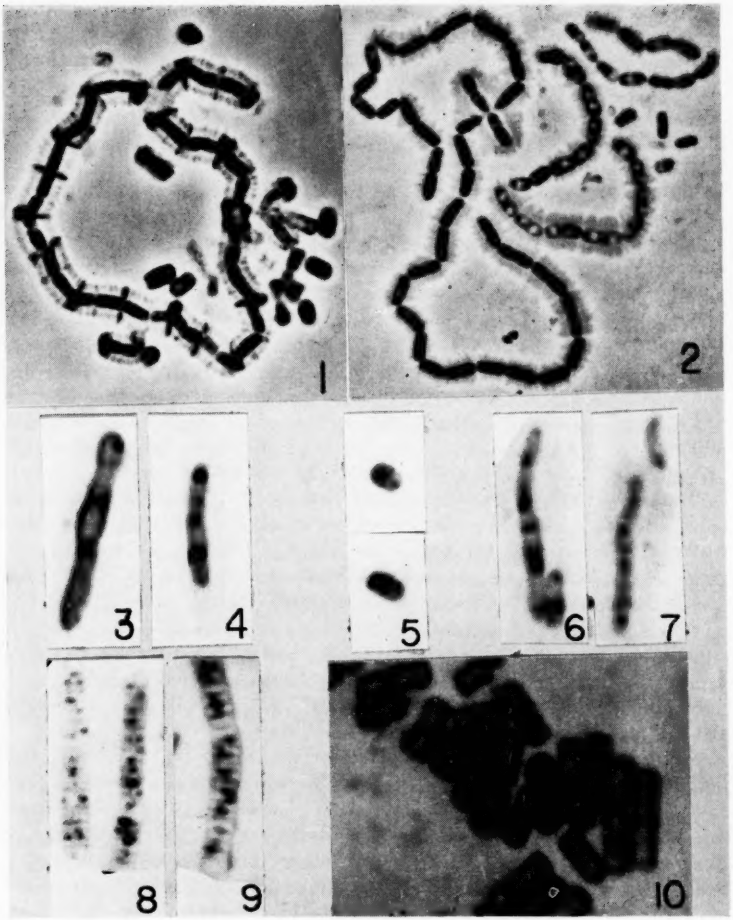


PLATE I

FIGURES 1 TO 10
LEGENDS

Pictures in Figures 1 to 10 hitherto unpublished.

Figures 1 and 2. "*Bacillus M.*" Magnification ×2500. Courtesy of Dr. J. Tomcsik, University of Basel.

FIG. 1. Aerated culture in Sauton medium. Secondary capsule production in the centrifuged sediment held three days at room temperature. Homologous antipolysaccharide antibody added to living bacilli; phase contrast photographs 15 min. later.

It may suffice, then, for the present reviewer to summarize essential conclusions and to cite a few very recent references. Bacterial flagella are true locomotor organelles which grow out from the protoplast, traversing the cell wall (30, 31, 32). Flagella may be single, may occur in tufts, or may occur around the periphery of the bacterial cell. Within the protoplast they may terminate in tiny spherules, for *Proteus vulgaris* (30) and *Vibrio cholerae* (30a) about 1000 Å in diameter. Single flagella have a strong tendency to aggregate in wavy bundles, by side to side apposition, and it is in such helical "tails"

Septa appear as dark streaks transverse to axis of chain.

FIG. 2. Bacilli prepared as in Fig. 1; treated with antipolypeptide serum. The sites of polysaccharide septa appear as gaps in the polypeptide matrix.

Figures 3 to 7. *Corynebacterium diphtheriae, mitis* strain A 9255. Magnification ×4850. Courtesy of John C. Davis. The metachromatic granules in these pictures are clearly not "entire, tiny constituent cells," as claimed by Bisset (54).

FIGURES 3 and 4. Grown 18 hr. at 37° on M. and E.[5] agar (168) containing 5 per cent human blood. Impression smear, unfixed, air dried. Stained after Ebel (10 sec. in 10 per cent lead nitrate, 10 sec. in 1 per cent ammonium sulfide); then after Hale (52) (5 min. in 1 per cent phosphomolybdic acid, 5 min. in unextracted 1 per cent aqueous methyl green). Mounted in Farrant's aqueous mounting medium. Metachromatic granules dark red, cell walls and septa purple.

FIG. 5. Coccoid phase; grown 24 hr. at 37° on M. and E. agar containing 5 per cent human blood. Cells stained 10 min. in 0.2 per cent crystal violet, 1 min. in 5 per cent citric acid; then after Hale (5 min. in 1 per cent phosphomolybdic acid, 3 min. in chloroform-extracted methyl green). Mounted in Farrant's medium. Metachromatic granules scarlet, cell walls and septa dark green.

FIGURES 6 and 7. Grown in M. and E. broth containing 5 per cent human blood serum 9 hr. at 37°. Cells fixed 1 min. over vapors of 2 per cent solution of OsO_4. Stained 10 sec. with Neisser's acidified methylene blue; placed 20 min. in saturated solution of $HgCl_2$ and 20 sec. in 0.1 per cent Victoria Blue B. Mounted in Farrant's medium. Metachromatic granules scarlet, cell walls and septa blue.

Figures 8 and 9. *Caryophanon latum*. Magnification ×3500. Courtesy of T. Sall.

FIG. 8. Grown 18 hr. at room temperature on cow dung agar. Air dried smear stained 10 sec. with 0.2 per cent crystal violet and placed for 60 sec. in 5 per cent citric acid. Mounted in Farrant's medium. Metachromatic granules purple.

FIG. 9. Culture and stain as in Fig. 8; then stained after Hale (5 min. in 1 per cent phosphomolybdic acid and 5 min. in 1 percent chloroform-extracted methyl green). Mounted in Farrant's medium. Metachromatic granules purple; cell walls and septa green.

FIG. 10. *Bacillus cereus*, strain p. 109. Magnification ×4850. Courtesy of John C. Davis. Grown 8½ hr. with aeration in M. and E. broth containing 5 per cent human blood serum. Air dried impression preparation stained 10 sec. in 0.2 per cent crystal violet, then 30 sec. in 5 per cent citric acid; stained after Hale (5 min. in 1 per cent phosphomolybdic acid, 1 min. in 1 per cent chloroform-extracted methyl green). Placed 1 min. in saturated HgCl to prevent subsequent decoloration and mounted in Farrant's medium. Metachromatic granules scarlet; cell walls and septa green.

[5] M. and E. refers to Morton and Engley.

that the flagella appear during locomotion. Weibull (33), by dissolving the cell walls of *Bacillus megaterium* with lysozyme, has obtained flagellated protoplasts.

Flagella may differ in different genera, structurally, and, for instance, in relation to serum agglutination (34). Thus in *Proteus*, *Bacillus*, *Salmonella*, and *Escherichia* (30, 33, 35, 36) the elementary flagella appear to be single threads; in *Vibrio metschnikovii* the flagellum comprises a sheath and central core (29); De Robertis & Franchi (37) ascribe to the flagella of *Bacillus brevis* a complex structure which, however, is questioned by Starr & Williams (38); the flagellum of a motile diphtheroid is described by Starr & Williams (38) as being composed of spirally wound fibers, arranged as a left-handed, triple-threaded helix, with a diameter of 190 Å. The flagella of certain large spirilla appear to be compound structures composed of a large number of individual fibers which arise in bundles from a single blepharoplast (30a).

X-ray diffraction studies of the elementary flagella of *Proteus* and of *Bacillus subtilis* have shown them to belong to Astbury's family of keratin-myosin-epidermin-fibrinogen fibrous proteins (39, 40, 41). Their diameter is about 120 Å, therefore less than that of the macromolecules of tobacco mosaic virus (150 Å). In fact examination of all available data leads Astbury (41) to regard them as monomolecular hairs or muscles. In a suspension of detached flagella some are in the α (folded) and some in the β (extended) configuration, and Astbury has transformed the α- into the β-form experimentally by compressing them in moist films. Astbury imagines a flagellum as "consisting of a long polypeptide loop: if then a wave of folding or unfolding ran down one side and up the other, it could be so arranged that the flagellum would wave back and forth." Astbury (41) thinks it probable that the initial stimulus and chemical energy for the contractile wave originate in the basal granules inside the protoplast. In comment Huggins (42) writes:

> Rotations about some of the bonds in the polypeptide chain (without alterations of the bond angles) should occur relatively easily. Neglecting hydrogen bond interactions, such rotations suffice to change a polypeptide chain from any one of several folded or helical types of structure to another or to an extended or nearly extended structure, such as is believed to exist in various fibrous proteins in the β-configuration. The energy needed for such changes is very small; it might well be supplied by alterations in the concentrations of certain ions at one end of the protein system. An excess of H^+, for instance, might favor one H-bond structure pattern; a deficiency, another.

Nakaya *et al.* (43) present evidence that the specific antigenic sites on flagella of Salmonellae occur in a mosaic arrangement. Presumably these sites could be determinant groupings along a polypeptide chain. The genetic determination of flagellation and of the specificities of flagellar antigens is reviewed by Zinder (8) and Stocker *et al.* (9). The facts of transduction of flagellar determinants, incidentally, afford powerful independent evidence of flagella as specific organelles. Burcik & Plankenhorn (44) describe fixing and mordanting procedures which make flagella readily demonstrable in the phase-contrast microscope.

The Cell Wall

A number of procedures appear in the recent literature which contribute to defining the positions of cell walls and intercellular septa. They include crushing (45, 46), plasmolysis and autolysis (47), special fixation and staining procedures (46 to 57), and electron microscopy (58 to 63). The protoplasts of bacterial cells are seen to be enclosed within well-defined external walls and intercellular septa whose thickness is well below the limit of light microscopic resolution. These walls are ordinarily stretched by the turgor of the protoplasts; on desiccation for electron microscopy the walls and septa shrink less than the protoplasts they contain and usually appear as delicate envelopes in which little or no fine structure is discerned. Measurements of cell wall thickness in electron micrographs are given by Knaysi, Hillier & Fabricant (59) for *Mycobacterium tuberculosis* var. *avium* as 230 Å; by Dawson (64) for *Staphylococcus aureus* as 150 to 200 Å; by Chapman & Hillier (60) in ultrathin sections of a rough variant of *Bacillus cereus* as 200 Å; by Birch-Andersen, Maaløe & Sjöstrand (61) in ultrathin sections of *Escherichia coli* B as 100 Å. According to Bisset (53), "The two main morphological types of bacteria, which correspond to the 'smooth' and 'rough' colony forms, divide by constriction of the cell wall and by the formation of cross walls respectively."

The components of the bacterial cell wall are arranged in ultramicroscopic mosaics of carbohydrate, protein, and lipid. Carbohydrate and protein components are usually antigenic and are the sites of interaction with immune antibodies. Such interactions are of critical importance in immunochemical phenomena (22, 23, 58). The finer gradations of serological typing are usually determined by the specificities of carbohydrate antigens of capsule or cell wall, less fine groupings by protein antigens; the type specific protein antigen M and the group specific carbohydrate C (65, 66) in the cell wall of *Streptococcus pyogenes* reverse this usual relationship.

Specific components or configurations of the mosaic in the surface of the cell wall also constitute the sites of attachment for bacteriophages. The specificities of the phage receptors may correspond to or be coarser or finer than the specificities of the antigenic components (cf. 67 to 72). Goebel & Jesaitis (69) have isolated the type specific somatic antigens, respectively, of *Shigella sonnei* Phase II and of a phage resistant mutant. These somatic antigens are also specific phage receptors; chemically they are lipomucoproteins. The antigen-receptors of the original and mutant strains differ with respect to the immunochemical and phage specificities and also the chemical composition of their lipocarbohydrate moieties. The lipocarbohydrate component of the specific phage receptor has been shown to possess the remarkable property of causing the corresponding phage particles to discharge their desoxyribonucleic acid contents (70, 71). Weidel (72) presents evidence that the basic structural framework of the cell wall of *E. coli* is lipoprotein.

The ultramicroscopic structure of bacterial cell walls has been investigated extensively by electron microscopy and by analytical chemical methods, following separation of the cell walls from the protoplasts by various mechanical means and lytic agents (47, 73 to 79). The separated cell walls

collapse against the supporting surface and shrink somewhat, but otherwise retain the approximate contours of the bacterial cell from which they came. The ultramicroscopic mosaic structure of the surface of the cell wall, clearly evidenced by immunochemical and phage-receptor analysis, is not rendered visible even in electron microscopic pictures. In cell walls lysed by phage, by heat, or by isoniazid, however, circular and elliptical segments have been observed (reviewed in 22; 77, Fig. 38). Filamentous extensions from the cell wall also have been observed following various preparative manipulations (22) and have been isolated by Weibull & Hedvall (80). A beautiful hexagonal pattern of macromolecular dimensions has been demonstrated by Houwink (81) in the cell walls of a *Spirillum*. The cell wall of baker's yeast cells has been shown by Northcote & Horne (82) to be composed of protein, lipid, and at least two polysaccharides, a mannan and a glucan. Electron microscopic and x-ray diffraction data on yeast cell walls by Houwink & Kreger (83) reveal about 75 per cent amorphous material permeating a fibrous meshwork. Bud scars are demonstrated (82, 83, 84).

Electron microscope studies of the cell walls of endospores of the genus *Bacillus* have been made by Knaysi, Baker & Hillier (85), Knaysi & Hillier (86), and Robinow (87, 88, 89). Enveloping the spores of *B. mycoides* two coats were found, "an inner, somewhat rigid one, and an outer, highly elastic one" (85). About the spores of *B. megaterium* (86) a single homogeneous appearing coat was found. Shed coats disintegrate, revealing that they consist "of a matrix of beaded threads held together by a cementing material; the diameter of a bead is about 120 Å, and that of the cross-section of the thread about 90 Å; the length of a thread varies considerably but is usually between 6×10^2 and 12×10^2 Å. Robinow (88) studied spores of *B. cereus* and *B. megaterium* in thin sections. The spore nucleus, which is central in the untreated spore, after treatment with mineral acid was shown to herniate through the spore wall, thus giving rise to the "extracytoplasmic" nucleus which has been so much discussed.

The Osmotic Barrier

The existence of an osmotic barrier regulating exchange between the protoplast and its external environment has been demonstrated for bacteria (90, 91) and for yeast (92). Analysis of phosphorus exchange across the osmotic barrier of *Micrococcus pyogenes* var. *aureus* suggests a mechanism of exchange-diffusion by which phosphate is shuttled across the barrier by carrier molecules (93, 94). Physico-chemical evidence has been marshalled (90) to indicate that the barrier is probably about 100 Å thick, and probably lies at the cytoplasmic surface of the protoplast. Intact protoplasts of *B. megaterium* have been isolated (33) in buffered sucrose solutions following lysis of the cell wall by lysozyme; these rounded protoplasts in endogenous respiration resemble the normal cells. From lysates of these protoplasts fixed in osmium tetroxide, Weibull (95) has isolated "ghosts" which may represent the cytoplasmic membranes, either intact or denatured. Associated with these mem-

branes were granules which (the reviewer suggests) may have been mitochondria, or fragments or subunits thereof, but concerning which no oxidation-reduction data were given.

The protoplast and its enveloping membrane are the structures rendered visible in ordinary bacteriological staining procedures. The role of the cytoplasmic membrane in the gram reaction is considered in detail by Stacey (96), Webb (97), Bartholomew & Mittwer (98), Fischer & Larose (99), and Mitchell & Moyle (100). Mitchell & Moyle (100) "suggest that a lipid-polyglycerophosphate-protein component may be a common structural feature of the cell envelopes of all gram positive organisms." In *Micrococcus pyogenes* (91), at least, part of the polyglycerophospho-compound is bound to the cell wall and part to the underlying lipoprotein membrane. Robinow & Murray (47) have demonstrated for dilute solutions of Victoria blues a selective staining affinity for the cytoplasmic membranes of gram positive species of *Bacillus*. Staining with this dye, after separation of cell wall and cytoplasmic membrane by plasmolysis, drying, or exposure to ether vapor, provided beautiful demonstrations of the cytoplasmic membrane.

The osmotic barrier at the surface of the bacterial protoplast has been estimated (90, 101) as at least an order of magnitude less in thickness than the limit of resolution of the light microscope, but within the resolving power of the electron microscope under suitable conditions. It may not be assumed, of course, that the osmotic barrier is necessarily identical with the morphological membrane which may be present at the surface of the protoplast. Knaysi, Baker & Hillier (85) and Knaysi & Baker (101) in electron micrographs of *B. mycoides* demonstrated very small bodies (0.08 by 0.1 μ to 0.13 by 0.17 μ), extremely thin, which they interpreted as in the cytoplasmic membrane. Robinow & Murray (47) published electron pictures of a thin membrane, distinct from the cell wall, stretched over the surface of disintegrating protoplasts of *B. cereus*. Electron pictures of cytoplasmic membranes in disrupted cells of *Pseudomonas denitrificans* and of *E. coli* have recently been published by Few (101a).

However Chapman & Hillier (60) in superb electron pictures of ultrathin sections demonstrating the centripetal growth of intercellular septa in a rough strain of *B. cereus*, were unable to show "any discrete entity that could be identified as the cytoplasmic membrane." These bacteria had been through a graded series of ethyl alcohols and the ultrathin sections had been floated on 30 per cent acetone solution. Internal evidence in the pictures caused the authors themselves to suggest the possibility of partial dissolution (of the contents of their "peripheral bodies"). Birch-Anderson, Maaløe & Sjöstrand (61) in an electron-microscopic study of thin sections of *E. coli*, note that

> when the fixed and washed cells were taken into 70 per cent ethanol . . . 5 to 8 per cent of the phosphorus of the cells went into solution. A substantial fraction of the phospholipid cell material thus remains soluble in ethanol after Os-fixation, and is never preserved in the dehydrated cells.

Alternatively it seems possible to the reviewer that the osmotic barrier described by Mitchell & Moyle (91, 100) in Chapman and Hillier's sections may have remained with the cell wall rather than shrinking with the protoplast. Sectioning of bacteria for electron microscopy obviously offers tremendous possibilities, but it seems clear that this most promising technique should not yet be expected to yield wholly interpretable results on all finer details.

Reduktionsorte, Corps Métaboliques, Mitochondria, Metabolitkörper, Chondrioïdes

Certain granules have been detected in the protoplasts of bacteria from the earliest years of bacteriology, e.g., the granules of tubercle bacilli described as spores by Koch in 1884 and the Babes-Ernst granules of *Corynebacterium diphtheriae*, and have been subject to a great diversity of interpretations. In the most recent years several streams of investigation, independent in origin but convergent in course, have united to indicate that the true nature of these granules is suggested by the above terms, all of which have been applied to them.

Bielig, Kausche & Haardick (102) demonstrated granular sites of reduction in the protoplasts of *E. coli* and *Salmonella typhosa*, using triphenyltetrazolium chloride as indicator, and showed that the properties of the reducing system corresponded to those of a flavoprotein. The sites of enzymatic reduction and the Feulgen-positive nucleoids were interpreted by these authors as constituting together a composite organelle, a view which they subsequently restated (103). The concept of a composite organelle, containing both ribosenucleic acid and nuclear desoxyribosenucleic acid was developed also on other grounds by Bringmann in a series of papers. Bringmann's Karyoid-hypothesis is summarized in Planta (104) and has been defended as late as 1953 (105). However, cogent evidence against the isotopic localization of RNA^2 and DNA^2 in a supposed organelle of composite function has been presented by Preuner & v. Prittwitz and Gaffron (106), by Tronnier (107), by Wintersheid & Mudd (108), and by Davis *et al.* (109). The subject is reviewed by Hahn (110) and in detail by Mudd (22) and Winkler (111); all three reviewers regard the hypothesis of a composite organelle with nuclear and mitochondrial functions as invalidated.

A clear cut demonstration of nuclear equivalents and metabolizing bodies (corps métaboliques) as separate organelles in the same bacterial cells was made by Kellenberger (112) before the International Congress of Electronmicroscopy in Paris in 1950. The proceedings of this Congress were not published until some years later. Mudd, Winterscheid & Brodie (113) independently reported to the German Society for Electron Microscopy in Hamburg in 1951 the recognition of cytoplasmic granules with properties corresponding to mitochondria. Subsequently detailed studies have been published, describing the properties of these cytoplasmic, metabolically active granules in *Mycobacterium* (62, 108, 114), *E. coli* (115, 116), *B. megaterium* (115), *M. cryophilus* (115), *Saccharomyces cerevisiae* (115, 117), *Salmonella typhosa*

(118), and *Proteus* (2, 119). These investigations have led to the following definition of the mitochondira of bacteria (2, 120):

These are defined as cytoplasmic granules, dense by electron microscopic and phase microscopic examination, possessing limiting membranes, and possessing coordinated systems of oxidative-reductive enzymes; they contain phospholipid and at least in certain cases metaphosphate. The bacterial mitochondria range from prolate ellipsoids and spheroids, similar in dimensions to the smaller mitochondria of mammalian cells to spheroids well below the limits of microscopic resolution. For the present at least, however, it seems better to refer to even the smaller cytoplasmic particles with the above morphological and biochemical properties as mitochondria rather than as microsomes. It should be noted also that appreciable functional differences among themselves have already been detected among bacterial mitochondria. In particular the cytochrome system may be absent or deficient. The term "mitochondria" in bacteria is thus used in a generic sense, with the expectation that specific differences will continue to be demonstrated between the several mitochondria of the same and of different bacterial cells and between these and the mitochondria of higher forms.

Cytoplasmic granules, under various modifying influences but with properties in accord with the above definition, have been described from Kellenberger's laboratory (121 to 124). The Swiss investigators have used as descriptive terms Metabolitkörper (121) and chondrioïdes (123, 124).

A third series of investigations has been concerned with enzymatic analyses of particulates obtained from bacteria. Georgi *et al.* (125) isolated a particulate enzymatically active fraction from a thermophilic bacterium. Under the phase contrast microscope only dense, spherical bodies were seen. When packed together by centrifuging, these had a bright red color attributable to cytochrome *c* and cytochrome oxidase. Burns & Militzer (126) demonstrated reduction of triphenyl tetrazolium chloride by the cytoplasmic granules both within the cells and after isolation. Enzymatic activities, demonstrated for the isolated particles as well as the intact cells, included succinoxidase, α-ketoglutaric oxidase, pyruvic oxidase, aconitase, isocitric dehydrogenase, and malic dehydrogenase. "The isocitric acid cycle appears in the cell granule."

Tissières (127) reported cell-free, particulate fractions from *Aerobacter aerogenes* which contain the succinic oxidase system. Vely & Asnis (128) studied particulate fractions from disintegrated cells of *E. coli* which contain succinoxidase, lactic and formic dehydrogenases, adenosine triphosphatase, and other activities; the granules, whether intracellular or extracellular, reduce triphenyl and neotetrazolium chlorides and stain similarly with citric acid-Sudan black B [Davis (129)].

The work and conclusions reviewed above have, of course, not been without critics. Bisset (130) wrote with reference to the electron scattering cytoplasmic granules of *M. tuberculosis* (114):

A somewhat different appearance is seen in Fig. 9, which represents the condition seen frequently in *M. tuberculosis*, when it is heat-fixed and stained. The cell contents

have shrunken away from the unstained cell wall, and appear as a string of granules. In a recent study, Mudd *et al.* have chosen to interpret these as "mitochondria."

Actually for the electron micrographs in question the bacteria had neither been heat-fixed nor stained. Electron micrographs of mycobacteria (22, 62, 108) demonstrate and differentiate cell walls and intercellular septa, protoplasts, and cytoplasmic granules with complete clarity. Comparison of Bisset's statement and the published pictures is invited. Moreover Knaysi (131), in living cells of *M. thamnopheos*, has demonstrated with the phase contrast microscope the division and fusion of the granules in question. Although Knaysi and the reviewer differ in their interpretation of the granules, their presence in the living cells is unquestionable.

The Bacteriaceae, according to Bisset (130) do not have crosswalls, but are unicellular and divide by constriction of the cell wall. "If bacteria of this type," Bisset (130) wrote, "are stained very briefly with aqueous basic dyes, the most obvious structures which appear are the growing points and cytoplasmic septa (Fig. 14). These have been confused with the nuclear structures by some observers and with mitochondria." Davis *et al.* (109) have stained cell walls, nuclei, and mitochondria differentially for the same cells of *S. typhosa*, and Hartman *et al.* (116) have demonstrated physiologically active mitochondria in the debris following phage lysis, again refuting Bisset's criticism.

Bisset (132) also claimed that Mudd and collaborators were confusing mitochondria with "complete protoplasts" of mycobacteria, "stainable areas at the growing tips and points of division of *Bacterium coli*" and "the basophilic elements of developing septa" of *B. megaterium*. The differentiation of protoplasts and mitochondria in *Mycobacterium* has already been considered (22, 62, 108, 114), as has the differentiation of cell walls, mitochondria, and nuclei in Bacteriaeceae (109, 116). In the case of *B. megaterium*, cell walls, intercellular septa, the mitotic nucleus, and mitochondria have also been stained differentially in the same cells (3, 4, and this chapter, Part II, Plate II, Figures 1 to 4). The correspondence of cell walls and complete and incomplete intercellular septa in the optical sections of DeLamater and in the electron micrographs of ultrathin sections by Chapman & Hillier (60) is very striking.

Finally, Bisset (133, 134) in discussing the Babes-Ernst granules of *C. diphtheriae*, which have been known and studied since before 1890, claimed that "the metachromatic granules are probably mere condensations of stainable material within the dried cell." "The 'characteristic morphology' of *C. diphtheriae* appears to be an artefact" (133). Such "artefacts," or perhaps the cell walls or septa, are supposed to have been misinterpreted as mitochondria (53, 130). The characteristic morphology of *C. diphtheriae* is not an artifact, as shown by the excellent study of Hewitt (135), comparing living cells of *Corynebacterium* observed with the phase contrast microscope

with cells stained for cell walls and septa and for nuclei. The admirable contribution of Winkler (111) may also be consulted in this connection. Plate I, Figures 3 to 7, show rods and coccoid forms of *C. diphtheriae* double stained for cells and septa and for metachromatic granules (136). The rods are multicellular, and even the coccoid forms have each a septum. However the walls and septa stained blue, green, or purple, and the metachromatic granules red, and the metachromatic granules are clearly within the protoplasts, which are themselves stained. The intercellular septa vary appreciably in thickness and definition, as shown also in Hewitt's (135) phase contrast pictures.

Certain other differences in interpretation that have arisen have been considered in detail in (2, 22). Brieger & Glauert (137), in a comprehensive study of the reproduction of *Mycobacterium tuberculosis* var. *avium*, using phase contrast and electron microscopy, note that the characteristic electron scattering granules are present both in the mycelial and the bacillary phases of reproduction. They write:

> In view of their position and arrangement they cannot be "nuclei" as suggested by Knaysi, Hillier and Fabricant (1950), and the term "nucleoids" (Ruska *et al.*, 1952) may also be misleading. According to Mudd, Winterscheid, DeLamater and Henderson (1951) these dense bodies are centres of energy metabolism and in function, biochemical nature and staining properties are similar to mitochondria.

The cytoplasmic, electron-scattering granules in various phases of development of *M. tuberculosis* var. *avium* are dealt with in further detail by Brieger, Cosslett, and Glauert (137a).

In addition Weibull (138) has questioned the interpretation of mitochondria, essentially on the ground that cells of *B. megaterium* (overstained with triphenyltetrazolium) show extragranular accumulations of formazan. This fact had been previously reported but insufficiently emphasized in our own studies (114, 115). Other redox indicators, i.e., neotetrazolium, blue tetrazolium, the Nadi reagent, and Janus green B, are not equally prone to cause extragranular staining. An excellent critique of cytochemical methods as applied to mitochondria of bacteria and yeast has been published by Hartman & Liu (117).

Bergersen (139) has also demonstrated granules of *B. megaterium* which

> are centres of oxidation-reduction processes involving cytochrome oxidase. They appear at the site of cell division before this takes place. . . . It is not surprising that the vigorous processes of bacterial cell division involve organelles which supply the requisite energy for the chemical syntheses involved.

With this the reviewer is in thorough agreement.

Metachromatic granules.—Refractile particles, often referred to as Babes-Ernst granules, metachromatic corpuscles, and volutin grains, occur in the cytoplasm of many species of bacteria, yeasts, molds, higher fungi, and algae (140 to 145). Provisional clarification, at least, of the highly-controversial

questions of the nature and function of these granules appears now to be possible.

Definitive data on the metachromatic granules of yeasts in particular are being obtained. Schmidt, Hecht & Thannhauser (146) studied yeast grown on phosphorus-poor medium and transferred to phosphorus-rich medium; the phosphate of the "P-enriched" yeast was precipitated with barium acetate at pH 4 and identified as metaphosphate. Wiame (147) presented analytic and metabolic data which indicate that the metachromatically staining materials in yeast are condensation products of phosphoric acid, designated generically as metaphosphate. That fraction of the metaphosphate which is insoluble in cold 10 per cent trichloroacetic acid is associated with proteinaceous material (148) and is rapidly and reversibly transformable into orthophosphate in the cell. Yeast grown in a phosphate-poor medium and transferred into a phosphate-rich medium containing potassium ions and a metabolizable substrate rapidly stores metaphosphate. When yeast which has previously accumulated metaphosphate is allowed to grow in a medium without phosphate, nucleic acid synthesis is accompanied at first by a disappearance of insoluble metaphosphate and then continues at the expense of the soluble metaphosphate (149). Wiame (147) finds further that the metachromatic corpuscles of diptheria bacilli behave in a manner similar to those of normal yeasts and yeasts charged with metachromatic polyphosphate.

Ebel (150) has extracted metaphosphates from baker's yeast; these comprise a series of anhydrides of phosphoric acid ranging from triphosphate to higher homologues of several thousand molecular weight. Ebel (151) was unable to obtain metaphosphates from staphylococci, colon bacilli, anthrax bacilli, or from mammalian organs. However a strain of *C. diphtheriae* yielded metaphosphates; under conditions in which the diphtheria bacilli were rich in metachromatic granules, the metaphosphate content was high; under conditions in which the bacteria were poor in metachromatic granules, metaphosphate content was low.

Yoshida & Yamataka (152) have made parallel studies of the respiration and the uptake of inorganic phosphate by yeast metabolizing on various substrates and in the presence of various inhibitors. In other experiments the rates of disappearance of inorganic phosphates were compared with Ba-insoluble, acid labile phosphate P (the metaphosphate fraction), and the amounts of metachromatic material. It was found that, by intact yeast cells as well as extracted juice, inorganic phosphate in the medium was transformed into higher metaphosphate, accompanied by the oxidation of glucose, alcohol, etc., or with fermentation.

Hartman & Liu (117) showed the principal but not exclusive site of accumulation of the metachromatic material in yeast cells to be the cytoplasmic granules which were characterized as mitochondria. Mitochondria were metachromatic both in wild type yeast cells and in a respirationally deficient mutant strain. Thus it seems to be established that in yeast cells at least meta-

chromatic granules may represent metaphosphate stored in cytoplasmic granules (mitochondria). These high-energy phosphates may liberate energy stepwise in response to metabolic needs (153). Storage of metaphosphate therefore would seem to represent an energy-accumulator mechanism (154, 155) in microorganisms, functionally equivalent to the storage of arginine phosphate and creatine phosphate in invertebrate and vertebrate muscle.

Bacterial metachromatic granules have been shown by a number of investigators beside Wiame and Ebel to involve metaphosphate. König & Winkler (156) demonstrated the identity of the volutin granules of *C. diphtheriae*, metachromatic by the Neisser's stain, with electron-scattering polar granules. Extraction of these granules yielded calcium and phosphate ions which the authors believed to have been freed from organic combination by the preparative process. Macary (157) identified the metachromatic granules of *Corynebacterium* with polyphosphates by a method of staining with salts of Co^{++} or Fe^{++}. Macary also reported the reduction of silver nitrate to metallic silver in the metachromatic corpuscles. These corpuscles were shown to be distinct from the bacterial nuclei. Minck & Minck (158) present evidence to indicate that the metaphosphates of the metachromatic granules of *C. diphtheriae* are associated with ribonucleic acid, from which they may be freed by the action of ribonuclease. Winkler (111) summarizes the evidence suggesting that the metachromatic (volutin) granules of *C. diphtheriae* are stored metaphosphate available on metabolic demand.

In *Mycobacterium tuberculosis* (hominis) and in *M. thamnopheos* Mudd and co-workers (114) and Winterscheid & Mudd (108) presented evidence that the metachromatic granules were associated with mitochondria. These mitochondria are intensely electron-scattering, spheroid to ovoid bodies which are readily volatilized by electron bombardment (114, 159, 160). Extracts yielded metaphosphate and, in model experiments, metaphosphate-ribonucleate mixtures were volatilized by electron bombardment in a manner like that of the metachromatic granules of the intact bacilli [Ruska *et al.* (160)].

The various data cited permit formulation of the hypothesis that the metachromatic granules of *Corynebacterium* and *Mycobacterium* are accumulations of metaphosphate formed with the aid of energy-yielding enzymatic reactions within the mitochondria (161).

Microsomes and Macromolecules

An excellent beginning has been made in disintegrating bacteria by lysis or by mechanical means and in carrying out ultracentrifugal analysis of their contents. Pardee *et al.* (162) and Schachman, Pardee & Stanier (163), in all extracts from several bacterial genera, found a component with a sedimentation constant of about 40 Svedberg units and a particle weight of about one million. Electron pictures showed these particles to be spheres of about 12 to 15 mμ diameter. The particle fraction, of which the 40 S particles are the

principal constituent, accounts for about a quarter of the nitrogen of the bacterial cell and contains the bulk of its PNA.[2] The particles contain considerable amounts of ribonuclease, catalase, apyrase, formic dehydrogenase, and succinodehydrogenase.

In the special case of the photosynthetic bacterium *Rhodospirillum rubrum* all of the pigments (bacteriochlorophyll and carotenoids) were found to be in "chromatophores." These were estimated to be spheroidal particles approximately 40 mμ in diameter; each bacterial cell was estimated to contain approximately 5000 chromatophores. Such chromatophores could not be recovered from the same bacteria grown chemoheterotrophically in the dark.

Ultracentrifugal patterns similar to those obtained by Schachman *et al.* (163) for *E. coli* and other species, except *R. rubrum*, have been published for *E. coli* B by Siegel, Singer & Wildman (164). In *E. coli* B infected with T_2 coliphage differences were found in the DNA fraction.

Weibull (95) has fractionated components from *B. megaterium* obtained by lysozyme treatment as well as by grinding. Again the distribution pattern of macromolecular components was very similar to that obtained by Schachman *et al.* (163). In addition, much larger granules believed to be of lipid material were found. Weibull also obtained "ghosts," membrane-like bodies with a variable number of granules attached. The cytochrome system was found in the "ghost" fraction, as well as an appreciable part of the ribonucleic acid.

Stanier, Gunsalus & Gunsalus (165) partitioned sonic extracts of *Pseudomonas fluorescens* into a coarse particle fraction, a fine particle fraction, and a soluble fraction. The enzymatic properties of the two particle fractions appeared to be identical. They contain "a considerable number of enzymes, including succinic, malic, and L-mandelic dehydrogenases, together with the complete transport system required to link the dehydrogenation of these substrates through the cytochromes to molecular oxygen."

Billen & Volkin (166) have fractionated the contents of a radiation resistant mutant of *E. coli* B, obtaining a fraction containing large particles and a fraction of small particles of 20 to 50 mμ diameter. The large particle fraction was three times more active than the small particle fraction as succinoxidase. However, the small particle fraction, and this fraction only, was able to synthesize adenosinetriphosphate by oxidative phosphorylation. The authors end their discussion with the sentence:

> It would be most interesting to determine what relation, if any, may exist between these particulate fractions obtained by ultracentrifugation of cell-free extracts and the mitochondria identified by vital staining of *E. coli* in the investigations of Mudd *et al.* (1951).

Actually these 20 to 50 mμ particles are about ten times smaller in diameter than the electron scattering particles found by Mudd *et al.* (115) and Hart-

man *et al.* (116) in the debris of *E. coli* following phage lysis and shown to give characteristic reactions for mitochondria with redox indicators, including Janus green B. The 20 to 50 mμ particles could conceivably contain fragments or subunits of the 200 to 400 mμ mitochondria. Dr. David Green informs the reviewer, moreover, that the succinoxidase function is very stable and oxidative phosphorylation very labile.

In none of the works cited above have bacterial nuclei appeared intact among the fractions obtained. The whole fascinating subject of bacterial cell particulates in relation to the cell physiology deserves much more thorough exploration. An excellent critique of the cytochemistry of the bacterial nucleus and cytoplasm has recently appeared [Vendrely (167)].

After completion of this Ms. an important contribution appeared on "Structure in the Bacterial Cell-Wall during Cell Division" (169).

LITERATURE CITED

1[6]. Mudd, S., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 3–7 (Penso, G., Ed., Charles C Thomas, Springfield, Ill. 181 pp., 1953)
2. Mudd, S., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 67–81 (1953)
3. DeLamater, E. D., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 108–35 (1953)
4. DeLamater, E. D., *Intern. Rev. Cytol.*, **2**, 158–77 (1953)
5. Leslie, J., *Phil. Mag.*, **51**, 411 (1818)
6. DeLamater, E. D., Hunter, M. E., Szybalski, W., Minsavage, E., and Bryson, V., *Riassunti Comunicazioni, 6th Intern. Congr. Microbiol.*, 48 (Rome, Italy, 1953)
7. Hartman, P. E., Payne, J. I., Mudd, S., and Liu, C., *Bact. Proc.*, 38 (1954)
8. Zinder, N. D., in *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 261–69 (1953)
9. Stocker, B. A. D., Zinder, N. D., and Lederberg, J., *J. Gen. Microbiol.*, **9**, 410–33 (1953)
10. Taylor, H. E., in *The Nature of the Bacterial Surface*, 123–43 (Miles, A. A., and Pirie, N. W., Eds., Charles C Thomas, Springfield, Ill. 179 pp., 1949)
11. Duguid, J. P., *J. Pathol. Bact.*, **63**, 673–85 (1951)
12. Tomcsik, J., *Ann. Rev. Biochem.*, **22**, 364–70 (1953)
13. Tomcsik, J., *Experientia*, **7**, 459–63 (1951)
14. Tomcsik, J., and Guex-Holzer, S., *Schweiz. Z. allgem. Pathol. Bakt.*, **14**, 515–22 (1951)
15. Tomcsik, J., and Guex-Holzer, S., *Schweiz. Z. allgem. Pathol. Bakt.*, **15**, 517–25 (1952)
16. Tomcsik, J., and Guex-Holzer, S., *Riassunti Comunicazioni, 6th Intern. Congr. Microbiol.*, **1**, 576–77 (Rome, Italy, 1953)
17. Tomcsik, J., and Guex-Holzer, S., *J. Gen. Microbiol.*, **10**, 317–24 (1954)
18. Tomcsik, J., and Guex-Holzer, S., *Schweiz. Z. allgem. Pathol. Bakt.*, **16**, 882–91 (1953)
19. Tomcsik, J., and Guex-Holzer, S., *J. Gen. Microbiol.*, **10**, 97–109 (1954)
20. Ivánovics, G., *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **159**, 178–87 (1953)
21. Ivánovics, G., and Horváth. S., *Acta Physiol. Acad. Sci. Hung.*, **4**, 175–86 401–8 (1953)
22. Mudd, S., *Intern. Rev. Cytol.*, **2**, 133–57 (1953)
23. Mudd, S., *Postgrad. Med.* **14**, 33–42 (1953)
24. Duguid, J. P., and Wilkinson, J. F., *J. Gen. Microbiol.*, **9**, 174–89 (1953)
25. Novelli, A., *Experientia*, **9**, 34 (1953)
26. McKinney, R. E., *J. Bact.*, **66**, 453–54 (1953)
27. Gerstley, L., and Morton, H. E., *J. Bact.*, **67**, 125–26 (1954)
28. Sterne, M., *Onderstepoort J. Vet. Research*, **25**, 51–52 (1952)
29. van Iterson, W., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 24–38 (1953)

[6] The Symposium on Bacterial Cytology (references 1, 2, 3, 29, 53, 89, 111, 124, 167) was published as a Supplement to the *Rendiconti dell' Instituto Superiore di Sanita* in Rome. The distributor for Europe and the British Empire, excepting Canada, is Blackwell Scientific Publications Ltd., Oxford, England; the distributor for the United States, Canada and South America is Charles C Thomas, Springfield, Illinois.

30. Houwink, A. L., and van Iterson, W., *Biochim. et Biophys. Acta*, **5**, 10–44 (1950)
30a. Grace, J. B., *J. Gen. Microbiol.*, **10**, 325–27 (1954)
31. Bisset, K. A., and Hale, C. M. F., *J. Gen. Microbiol.*, **5**, 150–54 (1951)
32. Bisset, K. A., *J. Gen. Microbiol.*, **5**, 155–58 (1951)
33. Weibull, C., *J. Bact.*, **66**, 688–95 (1953)
34. Pijper, A., Crocker, C. G., van der Walt, J. P., and Savage, N., *J. Bact.*, **65**, 636–41 (1953)
35. Weibull, C., and Hedvall, J., *Biochim. et Biophys. Acta*, **10**, 35–41 (1953)
36. Uchida, H., Sunakawa, S., and Fukumi, H., *Japan. J. Med. Sci. Biol.*, **5**, 351–55 (1952)
37. De Robertis, E., and Franchi, C. M., *Exptl. Cell Research*, **11**, 295–98 (1951)
38. Starr, M. P., and Williams, R. C., *J. Bact.*, **63**, 701–6 (1952)
39. Astbury, W. T., *Nature*, **167**, 880–81 (1951)
40. Weibull, C., *Nature*, **167**, 511–12 (1951)
41. Astbury, W. T., *The Harvey Lectures for 1950–51*, **46**, 3–44 (Charles C Thomas, Springfield, Ill., 313 pp., 1952)
42. Huggins, M. L. (Personal communication)
43. Nakaya, R., Uchida, H., and Fukumi, H., *Japan. J. Med. Sci. Biol.*, **5**, 467–73 (1952)
44. Burcik, E., and Plankenhorn, B., *Arch. Mikrobiol.*, **19**, 435–37 (1953)
45. Murray, R. G. E., and Robinow, C. F., *J. Bact.*, **63**, 298–300 (1952)
46. Welshimer, H. J., *J. Bact.*, **66**, 112–17 (1953)
47. Robinow, C. F., and Murray, R. G. E., *Exptl. Cell Research*, **4**, 390–407 (1953)
48. Cassell, W. A., *J. Bact.*, **62**, 239–41 (1951)
49. Chance, H. L., *Stain Technol.*, **28**, 205–7 (1953)
50. Chance, H. L., *J. Bact.*, **65**, 593–95 (1953)
51. Chance, H. L., *J. Bact.*, **66**, 239–40 (1953)
52. Hale, C. M. F., *Lab. Practice*, **2**, 115–16 (1953)
53. Bisset, K. A., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 9–23 (1953)
54. Bisset, K. A., and Hale, C. M. F., *Exptl. Cell Research*, **5**, 449–54 (1953)
55. Bisset, K. A., *J. Bact.*, **67**, 41–44 (1954)
56. Webb, R. B., and Clark, J. B., *J. Bact.*, **67**, 94–97 (1954)
57. Webb, R. B., *J. Bact.*, **67**, 252–53 (1954)
58. Mudd, S., and Anderson, T. F., *J. Am. Med. Assoc.*, **126**, 561–71 (1944)
59. Knaysi, G., Hillier, J., and Fabricant, C., *J. Bact.*, **60**, 423–47 (1950)
60. Chapman, G. B., and Hillier, J., *J. Bact.*, **66**, 362–73 (1953)
61. Birch-Andersen, A., Maaløe, O., and Sjöstrand, F. S., *Biochim. et Biophys. Acta*, **12**, 395–400 (1953)
62. Mudd, S., and Winterscheid, L. C., *Exptl. Cell Research*, **5**, 251–54 (1953)
63. Mudd, S., Hillier, J., Beutner, E. H., and Hartman, P. E., *Biochem. et Biophys. Acta*, **10**, 153–79 (1953)
64. Dawson, I. M., in *The Nature of the Bacterial Surface*, 119–20 (Miles, A. A., and Pirie, N. W., Eds., Charles C Thomas, Springfield, Ill., 179 pp., 1949)
65. McCarty, M., *J. Exptl. Med.*, **96**, 555–80 (1952)
66. Salton, M. R. J., *Biochim. et Biophys. Acta*, **10**, 512–23 (1953)
67. Weidel, W., *Z. Naturforsch.*, **6b**, 251–59 (1951)
68. Beumer, J., *Ann. Inst. Pasteur*, **84**, 15–26 (1953)
69. Goebel, W. F., and Jesaitis, M. A., *Ann. Inst. Pasteur*, **84**, 66–72 (1953)
70. Jesaitis, M. A., and Goebel, W. F., *Nature*, **172**, 622–27 (1953)

71. Jesaitis, M. A., and Goebel, W. F., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 205–8 (1953)
72. Weidel, W., *Ann. Inst. Pasteur*, **84,** 60–65 (1953)
73. Nermut, M., *Lékařské Listy*, **5,** 589–93 (1950)
74. Salton, M. R. J., and Horne, R. W., *Biochim. et Biophys. Acta*, **7,** 19–42, 177–97 (1951)
75. Kern, R. A., Kingkade, M. J., Kern, S. F., and Behrens, O. K., *J. Bact.*, **61,** 171–78 (1951)
76. Hotchin, J. E., Dawson, I. M., and Elford, W. J., *Brit. J. Exptl. Pathol.*, **33,** 177–82 (1952)
77. Angelico, R., Calo, A., D'Amore, A., Mariani, A., Mariani-Marelli, O., and Scanga, F., *Rend. ist. super. sanità*, **15,** 627–703 (1952)
78. Salton, M. R. J., *J. Gen. Microbiol.*, **9,** 512–23 (1953)
79. Farrant, J. L., and Rountree, P. M., *J. Gen. Microbiol.*, **9,** 288–92 (1953)
80. Weibull, C., and Hedvall, J., *Biochim. et Biophys. Acta*, **10,** 35–41 (1953)
81. Houwink, A. L., *Biochim. et Biophys. Acta*, **10,** 360–66 (1953)
82. Northcote, D. H., and Horne, R. W., *Biochem. J.* (*London*), **51,** 232–36 (1952)
83. Houwink, A. L., and Kreger, D. R., *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **19,** 1–24 (1953)
84. Bartholomew, J. W., and Mittwer, T., *J. Bact.*, **65,** 272–75 (1953)
85. Knaysi, G., Baker, R. F., and Hillier, J., *J. Bact.*, **53,** 525–37 (1947)
86. Knaysi, G., and Hillier, J., *J. Bact.*, **57,** 23–29 (1949)
87. Robinow, C. F., *J. Gen. Microbiol.*, **5,** 439–57 (1951)
88. Robinow, C. F., *J. Bact.*, **66,** 300–11 (1953)
89. Robinow, C. F., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità.*, 139–43 (1953)
90. Mitchell, P., in *The Nature of the Bacterial Surface*, **55–75** (Miles, A. A., and Pirie, N. W., Eds., Charles C Thomas, Springfield, Ill., 179 pp., 1949)
91. Mitchell, P., and Moyle, J., *J. Gen. Microbiol.*, **5,** 981–92 (1951)
92. Conway, E. L., and Downey, M., *Biochem. J.* (*London*), **47,** 347–55 (1950)
93. Mitchell, P., and Moyle, J. M., *J. Gen. Microbiol.*, **9,** 257–72 (1953)
94. Mitchell, P., *J. Gen. Microbiol.*, **9,** 273–87 (1953)
95. Weibull, C., *J. Bact.*, **66,** 696–702 (1953)
96. Stacey, M., in *The Nature of the Bacterial Surface*, 29–54 (Miles, A. A., and Pirie, N. W., Eds., Charles C Thomas, Springfield, Ill., 179 pp., 1949)
97. Webb, M., *Research* (*London*), **3,** 113–120 (1950)
98. Bartholomew, J. W., and Mittwer, T., *Bact. Revs.*, **16,** 1–29 (1952)
99. Fischer, R., and Larose, P., *Can. J. Med. Sci.*, **30,** 86–105 (1952)
100. Mitchell, P., and Moyle, J. M., *J. Gen. Microbiol.*, **10,** No. 3 (1954)
101. Knaysi, G., and Baker, R. F., *J. Bact.*, **53,** 539–53 (1947)
101a. Few, A. V., *J. Gen. Microbiol.*, **10,** 304–8 (1954)
102. Bielig, H.-J., Kausche, G. A., and Haardick, H., *Z. Naturforsch.*, **4b,** 80–91 (1949)
103. Bielig, H.-J., Kausche, G. A., and Haardick, H., *Naturwissenschaften*, **39,** 354 (1952)
104. Bringmann, G., *Planta*, **40,** 398–406 (1952)
105. Bringmann, G., *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **159,** 424–27 (1953)
106. Preuner, R., and v. Prittwitz und Gaffron, J., *Zentr. Bakt. Parasitenk., Abt. I. Orig.*, **157,** 244–50 (1951)
107. Tronnier, E. A., *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **159,** 213–16 (1953)
108. Winterscheid, L. C., and Mudd, S., *Am. Rev. Tuberc.*, **67,** 59–73 (1953)

109. Davis, J. C., Winterscheid, L. C., Hartman, P. E., and Mudd, S., *J. Histochem. and Cytochem.*, **1**, 123–37 (1953)
110. Hahn, F. E., *Naturwissenschaften*, **39**, 527–28 (1952)
111. Winkler, A., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità.*, 39–44 (1953)
112. Kellenberger, E., Presented at Ier Congrès Intern. Microscopie Electronique (Paris, France, 1950); *Rev. opt.*, 727–32 (1953)
113. Mudd, S., Winterscheid, L. C., and Brodie, A. F., *Physik. Verhandl., Physik. Blätter*, **4**, 79–80 (1951)
114. Mudd, S., Winterscheid, L. C., DeLamater, E. D., and Henderson, H. J., *J. Bact.*, **62**, 459–75 (1951)
115. Mudd, S., Brodie, A. F., Winterscheid, L. C., Hartman, P. E., Beutner, E. H., and McLean, R. A., *J. Bact.*, **62**, 729–39 (1951)
116. Hartman, P. E., Mudd, S., Hillier, J., and Beutner, E. H., *J. Bact.*, **65**, 706–14 (1953)
117. Hartman, P. E., and Liu, C., *J. Bact.*, **67**, 77–85 (1954)
118. Davis, J. C., Winterscheid, L. C., Hartman, P. E., and Mudd, S., *J. Histochem. and Cytochem.*, **1**, 123–37 (1953)
119. Sorouri, P., and Mudd, S., *Indian J. Med. Research*, **41**, 49–53 (1953)
120. Mudd, S., *J. Histochem. and Cytochem.*, **1**, 248–53 (1953)
121. Kellenberger, E., *Z. wissensch. Mikroskop.*, **60**, 408–18 (1952)
122. Steinberg, B., *Schweiz. Z. allgem. Pathol. Bakt.*, **15**, 432–43 (1952)
123. Kellenberger, E., and Huber, L., *Experientia*, **9**, 289–91 (1953)
124. Kellenberger, E., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super sanità*, 45–66 (1953)
125. Georgi, C. E., Militzer, W., Burns, L., and Heotis, J., *Proc. Soc. Exptl. Biol. Med.*, **76**, 598–601 (1951)
126. Burns, L., and Militzer, W., *Proc. Soc. Exptl. Biol. Med.*, **82**, 411–13 (1953)
127. Tissières, A., *Nature*, **169**, 880 (1952)
128. Vely, V., and Asnis, R. E. (Unpublished data)
129. Davis, J. C. (Unpublished data)
130. Bisset, K. A., *Exptl. Cell Research*, **3**, 681–88 (1952)
131. Knaysi, G., *J. Bact.*, **64**, 859–64 (1952)
132. Bisset, K. A., *J. Gen. Microbiol.*, **8**, 50–57 (1953)
133. Bisset, K. A., *J. Gen. Microbiol.*, **3**, 93–96 (1949)
134. Bisset, K. A., *Intern. Rev. Cytol.*, **1**, 93–106 (1952)
135. Hewitt, L. F., *J. Gen. Microbiol.*, **5**, 287–92 (1951)
136. Davis, J. C., and Mudd, S. (In preparation)
137. Brieger, E. M., and Glauert, A. M., *Tubercle*, **34**, 128–38 (1953)
137a. Brieger, E. M., Cosslett, V. E., and Glauert, A. M., *J. Gen. Microbiol.*, **10**, 294–303 (1954)
138. Weibull, C., *J. Bact.*, **66**, 137–39 (1953)
139. Bergersen, F. J., *J. Gen. Microbiol.*, **9**, 26–29 (1953)
140. Lewis, I. M., *Bact. Revs.*, **5**, 181–230 (1941)
141. Dubos, R., *The Bacterial Cell*, 22, 24 (Harvard University Press, Cambridge, Mass., 460 pp., 1945)
142. Nagel, L., *Botan. Rev.*, **14**, 174–84 (1948)
143. Knaysi, G., *Elements of Bacterial Cytology*, 2nd ed., 195–96 (Comstock Publishing Co., Ithaca, N. Y., 375 pp., 1951)
144. Jensen, H. L., *Ann. Rev. Microbiol.*, **6**, 77–90 (1952)

145. Malmgren, H., *Svensk Kem. Tidskr.*, **64**, 27–39 (1952)
146. Schmidt, G., Hecht, L., and Thannhauser, S. J., *J. Biol. Chem.*, **166**, 775–76 (1946)
147. Wiame, J. M., *Biochim. et Biophys. Acta*, **1**, 234–55 (1947)
148. Juni, E., Kamen, M. D., Spiegelman, S., and Wiame, J. M., *Nature*, **160**, 717–18 (1947)
149. Wiame, J. M., *J. Biol. Chem.*, **178**, 919–29 (1949)
150. Ebel, J. P., *Bull. soc. chim. biol.*, **34**, 321–35 (1952)
151. Ebel, J. P., *Bull. soc. chim. biol.*, **34**, 491–97 (1952)
152. Yoshida, A., and Yamataka, A., *J. Biochem. (Japan)*, **40**, 85–94 (1953)
153. Ebel, J. P., *Bull. soc. chim. biol.*, **34**, 498–505 (1952)
154. Lipmann, F., *Advances in Enzymol.*, **1**, 99–162 (1941)
155. Schmidt, G., in *Phosphorus Metabolism*, **1**, 443–75 (McElroy, W. D., and Glass, B., Eds., Johns Hopkins Press, Baltimore, Md., 762 pp., 1951)
156. König, H., and Winkler, A., *Naturwissenschaften*, **35**, 136–44 (1948)
157. Macary, J., *Ann. Inst. Pasteur*, **81**, 657–62 (1951)
158. Minck, R., and Minck, A., *Compt. Rend.*, **228**, 1313–15 (1949)
159. Lembke, A., and Ruska, H., *Klin. Wochnschr.*, **19**, 217–20 (1940)
160. Ruska, H., Bringmann, G., Neckel, I., and Schuster, G., *Z. wiss. Mikroskop.*, **60**, 425–47 (1952)
161. Davis, J. C., and Mudd, S., *Federation Proc.*, **13**, 427 (1954)
162. Pardee, A. B., Schachman, H. K., and Stanier, R. Y., *Nature*, **169**, 282 (1952)
163. Schachman, H. K., Pardee, A. B., and Stanier, R. Y., *Arch. Biochem. Biophys.*, **38**, 245–260 (1952)
164. Siegel, A., Singer, S. J. and Wildman, S. G.: *Arch. Biochem. and Biophys.*, **41**, 278–93 (1952)
165. Stanier, R. Y., Gunsalus, I. C., and Gunsalus, C. F., *J. Bact.*, **66**, 543–47 (1953)
166. Billen, D., and Volkin, E., *J. Bact.*, **67**, 191–97 (1954)
167. Vendrely, R., in *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super sanità*, 82–107 (1953)
168. Morton, H. E., and Engley, F. B., Jr., *J. Bact.*, **49**, 245–55 (1945)
169. Dawson, I. M. and Stern, H., *Biochim. et Biophys. Acta*, **13**, 31–40 (1954)

CYTOLOGY OF BACTERIA[1,2]

PART II: THE BACTERIAL NUCLEUS[3]

By Edward D. DeLamater

Section of Cytology and Cytochemistry, Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

The purpose of this review is to formulate, if possible, a conceptual statement of the bacterial nucleus on the basis of current knowledge. Since there have been several reviews and general articles as well as texts presented in the field during the past several years, including those of Bisset (1, 6), Lewis (2), Knaysi (3, 4), Piekarski (5), Robinow (7), DeLamater (8, 9), and DeLamater, Hunter & Mudd (10), there is no need to recapitulate the history, except with reference to special aspects of the subject.

Basic concepts of bacterial nuclear structures current among various investigators.—The original observations of Stille (11) and Piekarski (12, 13, 14) have been effectively elaborated by Robinow (7, 15, 16), and subsequently followed in general and extended by Klieneberger-Nobel (17), Tulasne (18), Duguid (19), Flewett (20), and Kellenberger (21, 22), among many others (2 to 9). The observations made possible by the methods then at hand permit consideration of the bacterial nucleus essentially as follows. The bacterial nucleus has been considered to be comprised of a dumbbell-shaped rod lying transverse to the axis of the bacterial cell. This rod enlarges and subsequently divides longitudinally through its long axis; the two daughter chromatinic bodies separate, migrate apart, and subsequently undergo a similar divisional process. As a working hypothesis, it was presumed that the nuclear body, or "nucleoid," is made up of a single "chromosome" and represents the nuclear apparatus of the bacterial cell. Although it has been specifically stated by some (6, 23) to represent a different order of nuclear apparatus than found in larger cells, others (7, 16, 18) have continued to approach the problem as not resolved. That this bacterial "nucleoid" is probably more complicated than a single bar-like body was indicated early by Robinow (7) in his photographs of the Welch bacillus, in which the nuclear structures seem to be composed of at least three discrete bodies, and by Kellenberger (22) in *Escherichia coli*.

The basic structure, as described, has been demonstrated in both gram positive and gram negative rod-like bacteria, including *Bacillus megaterium*, *Bacillus cereus*, *Escherichia coli*, and *Caryophanon* (24), among many others.

[1] The survey of literature pertaining to this review was completed in February, 1954.

[2] The following abbreviations have been used in this chapter: RNA (ribonucleic acid); DNA (desoxyribonucleic acid).

[3] This study was supported in part by a grant from the Atomic Energy Commission, Contract No. AT (30-1)-1341.

No adjuvant divisional apparatus or mechanism was described in connection with the chromatinic bodies. Although additional structures apparently connected with or seen as a part of the nucleus were noted and photographed, they were not interpreted, apparently because of size and insufficient resolution (7). It has been tacitly assumed, mainly on the basis of genetic continuity, that the division must be equational (134), although occasionally referred to as amitotic (31). It may, perhaps, be injected here that the suggestion of, or the demonstration of a true mitotic process in the nuclei of bacteria would in no way detract from the superlative basic efforts of Robinow (7), Murray (32), Kellenberger (21, 22), Piekarski (5), and a host of others.

Despite the rather clear delineation of these chromatinic bodies as representing the bacterial nucleus, confusion has persisted between these structures and other components within the bacterial cell (25, 26, 30). Metachromatic granules particularly have been invoked as belonging to the nuclear apparatus. On the basis of the work by Kellenberger (22), Mudd and his associates (see 28), Winkler (27), and Tronnier (59), it seems clear that these intracellular bodies have now been differentiated as distinct organelles (see Part I by Mudd of this review).

The differentiation has been made on the basis of the simultaneous staining in the same cell of the two different types of organelles, demonstrating clearly that they cannot be the same structures. This definition of intracellular identity of organelles by simultaneous differential staining is quite distinct from the staining of two parallel preparations by different methods and the attempt to draw conclusions from these (33, 34). More will be said later concerning this in regard to the definition of spindle elements and cross walls.

Methods

An excellent review of histochemical methods as applied to bacteria recently has been given by Vendrely (35). Demonstration of the bacterial nucleus originally depended upon the development of a method in which acid hydrolysis was utilized for the purpose of elimination of the cytoplasmic density due to the constituent RNA.[2] This procedure, derived from the Feulgen reaction, was developed by Piekarski (12), and subsequently by Robinow (7), and others (10). The Piekarski-Robinow technique differs from the Feulgen procedure in that the Giemsa mixture is substituted for the Schiff reagent. The Giemsa mixture as a chemical reagent lacks the chemical specificity of the Schiff reagent (9) because of the complexity of the mixture of dyes present in it. It seems likely that adsorption plays a large part in the staining of the nuclear bodies, especially since these nuclear bodies are much increased in size by this technique (36).

More recently, single basic dyes have been substituted for the Giemsa stain. Smith (37) has substituted basic fuchsin; and he followed DeLamater (38) in the use of formaldehyde as a mordant. DeLamater (39) has used individual thiazin dyes as substitutes for the Giemsa stain in the staining of *Micrococcus* cells.

Cassel (41) effectively modified the hydrolysis procedure by substituting perchloric acid for hydrochloric acid. He subsequently stained the bacterial nuclei with basic fuchsin with an excellent differentiation of the nuclear structures. The efficacy of perchloric acid as hydrolyzing agent in the classical Feulgen procedure has been demonstrated by di Steffano (42). Welsch & Nikoul (29) substituted Na_2HPO_4 and NaOH treatment for the acid hydrolysis with excellent results, while the orcein method has been shown to be useful in demonstrating the chromosomal material of bacteria (128).

DeLamater (8, 39) further modified the procedure by substituting a thiazin dye, azure A or thionin, to which sulfurous acid was added as in the Schiff reagent used in the Feulgen procedure. Precedent for this substitution of thiazin or oxazin dyes in place of the rosaniline series in the manufacture of the Schiff reagent had been established by the excellent work of Östergren (43), and by Atwood & Ornstein (44), and later published by Heubschman (45). By this latter technique a degree of visualization of the nuclear structure of bacteria has been accomplished which had not been previously achieved. In addition to the modification of the staining procedure, DeLamater (39, 46) instituted the use of a freezing-dehydration technique in freezing alcohol by which permanent, undistorted preparations of bacterial cells were made possible. Although Bisset (33, 34, 47 to 53) has repeatedly denied the efficacy of this method, its usefulness is well-substantiated in the field of general cytology (9) as shown by Bell (54). The demonstration by Clark, Galyen & Webb (129) that various organic solvents affect both the shape and stainability of the bacterial nucleus, is not pertinent here, despite argument to the contrary (34).

Tulasne & Vendrely (55), Leonardi (56), and Keigler & Smith (57) have applied the use of ribonuclease for the purpose of eliminating the cytoplasmic density in order better to visualize the nuclear structures. Hartman & Payne (58) applied the staining procedure of May-Grünwald to the problem of differentiating bacterial nuclei and cytoplasm with success, and have amplified the excellent work of Tronnier (59). Chance (60) has attempted to develop a stain for bacterial nuclei based pragmatically upon an unknown reduction reaction produced ostensibly by some intracellular component. Recently an effort has been made by Quintini & Tchan (61), Cutinelli & Lamanna (62, 63), Califano (64), Caselli (65), Welshimer (66), Weibull (67), and others (115) to study the cytologic effects of lysozyme on the bacterial cell. It would appear that the nucleus remains unaffected by this reagent, provided autolysis is prevented. Further effort to define the structure of isolated nuclei by these and other (70) means is obviously indicated. Lembke (69), among others (68), used the electron microscope in his effort to visualize the effects of lysozyme. Study of the intrinsic structure of the bacterial nucleus, however, has not so far been made by these means.

Many authors, including Kellenberger (21, 22), Hillier, Knaysi & Baker (71), among others (72, 73, 74, 87), have applied the electron microscope to the elucidation of the structure of the bacterial nucleus without outstanding

success. They have, however, laid the foundation for more penetrating studies. The replica methods used by Kellenberger are very promising (21, 22). Chapman & Hillier (75), and Birch-Andersen, Maaløe & Sjöstrand (76) have recently applied ultra thin-sectioning to the bacterial cell. The problems of interpretation of results obtained with this instrument are difficult and many. Bradfield (143) in a magnificent piece of work has developed a modified Feulgen procedure for electron microscopy, using the aldehydes liberated by HCl hydrolysis to precipitate metallic silver (from ammoniacal silver nitrate) on (or in) the chromosomes. The author used this method on thin sections cut from material fixed in osmium vapors and embedded in methacrylate. In Chapman & Hillier's photographs the nuclear structures appear as somewhat strung-out, indefinite areas in the center of the cell, whereas in Birch-Andersen, Maaløe, & Sjöstrand's work the nuclei appear as rounded vesicles with a central aggregation of what they believe to be chromatinic material. It may be questioned whether the results of Birch-Andersen, Maaløe & Sjöstrand are not dependent on coagulation artifacts produced by too rapid alcoholic dehydration at room temperature (46). An excellent beginning has been made, however, on a series of very difficult problems.

Studies on methods deserve consideration here, since they represent an effort to approach an extremely difficult group of problems from the technical point of view. In all of this work cited the realization that the ultimate solution of the problems will depend upon the development and application of adequate methods yielding interpretable results seems to be tacitly understood.

Bisset has been critical of both the methods used and the results obtained by DeLamater. He has repeatedly made the statement that no controls were ever used (33, 34, 47, 48), and ignores (34) both the original paper of Blank, McCarthy & DeLamater (46), in which controls were cited with photographic evidence, and Robinow's comparative photographs (36) (presented at the International Congress of Microbiology by DeLamater in Rome, 1953) of the same cells as they appear in water mounts and subsequently as they appear in the dehydrated state. Bisset repeatedly states (33, 34, 47 to 51) that DeLamater's methods produce distortion and shrinkage and lack specificity, despite the earlier and independent work of Östergren (43), Atwood & Ornstein (44), and Heubschman (45) on the staining method, indicating a marked specificity if the chemical reactions involved are controlled (9, 54, 77). Bisset's objections (34, 51, 53) to the method are adequately answered by reference to those (54) with extensive experience in freezing-dehydration procedures, and by the following statement: "If the procedure is properly handled according to the prescribed recipe, it is impossible to obtain the results which Bisset describes" (9). In short, the charge that severe distortion and shrinkage are produced by the freezing technique cannot be substantiated in fact, nor has it proven to be the experience of Robinow (78), or of Wenrich (79), among others (80, 81). Robinow (78, 82) has further stated that the objects which Bisset decries as produced by distortion and shrinkage

in DeLamater's procedure are present in the water mounts and, therefore, cannot be produced by the freezing-dehydration method.

Bisset (52) elaborates in general that fixation is "dangerous and valueless," and considers that DeLamater has used unsuitable fixation methods. This is interesting in view of the fact that most workers in the field have used OsO_4. The use of Bouin's fluid, formaldehyde, alcohol as fixatives, as well as osmic acid, has been specifically condemned (52). This general condemnation of fixation cannot be defended. Fixation has been known since the days of Heidenhain to be necessary to the proper staining of chromosomes. Robinow (7, 15) has long used both osmium and Bouin's fiuid effectively on the bacterial cell; and Murray's recent work (32), although it shows clearly that different fixatives have differing effects on structures such as the nucleus, which ultimately must be evaluated and understood, does not support Bisset's contentions. Neither, it is believed, does the work of Birch-Andersen, Maaløe & Sjöstrand, as cited by Bisset, support this author's contentions, since Birch-Andersen, Maaløe & Sjöstrand used OsO_4 and did a one-step dehydration in alcohol at room temperature. DeLamater (39) also obtained coagulation artifacts with alcoholic dehydration at room temperature (46), and his conditions were less rigorous than Birch-Andersen's. The observations of Murray & Robinow (113) on the shrinkage of the protoplast produced in broken cells fixed in methanol-formalin, do not support the contention that "fixation is valueless." Unfractured cells showed no shrinkage.

Cassel (83) developed a procedure for the simultaneous demonstration of cell walls and bacterial nuclei. Both structures are stained red by this technique. Hunter [see DeLamater (9)] further refined this method by applying the tannic acid mordanting of Cassel to cells stained by DeLamater's azure A-sulfurous acid technique, subsequently staining them with basic fuchsin. The nuclei and associated structures are stained blue, and the cell walls are stained red (9, 36). Such simultaneous staining of different components of the same bacterial cell makes it readily possible to differentiate between them, whereas parallel preparations by different methods may lead to error in interpretation, since there can be no absolute assurance in two different preparations that the same structure is not visualized differently. Simultaneous differential staining of structures within the same cell has been used for many years as a replacement, both ideologically and practically, for the concept expressed by Bisset (33) that "parallel preparations with wall stains and with nuclear stains are a 'sine qua non' for the definition of intracellular structures." Bisset has not admitted the use of simultaneous staining by either Cassel's or by Hunter's combination of Cassel's and DeLamater's procedures. From the work of Kellenberger (22), Mudd and his associates (28), and Winkler (27), among others (59), it would appear obvious that confusion can occur by the use of parallel preparations (26) and that this confusion can be resolved only by the application of simultaneous procedures to the same cell, as was done by these authors (22, 27, 28, 59). Hale's wall stain (135), though useful, is not the panacea Bisset (34) would make of it, since it cannot be used

in a procedure for simultaneous staining of wall and nuclei (136).

Hoffman (23), after studying cells treated with ribonuclease and hydrochloric acid hydrolysis, considers that "the present techniques are not adequate for disclosing DNA (desoxyribonucleic acid) in the bacterial cell." This approach, especially since no new avenues are suggested, appears to the author as unnecessarily nihilistic.

Clifton & Ehrhard (84) applied the use of phase contrast to the study of the nucleus of *Bacillus anthracis*. Tulasne (85), Stempen (86), and Knöll & Zapf (102) have likewise studied the bacterial nucleus in this way. These authors agree that the light areas in the cells seen in dark-ground phase correspond to stained nuclei. According to Clifton & Ehrhard, the chromatinic body divisions in living cells were easily followed and occurred on lines perpendicular to the axis of the cell. These authors further state that chromatinic bodies are made up of parallel strands which increase in number as the chromatinic body increases in size. The number of strands seen in the chromatinic bodies ranges from two to six or more. None of the chromatinic bodies observed with the phase contrast microscope presented the dumbbell shape described in the stained preparations. This concurs with the observation of Stempen (86). Clifton & Ehrhard further state that the structures are definitely round, that the outline is not sharp, that there is a dense core with a less dense periphery, and that this would correspond to the structure of a sphere. They could observe no membrane, but the area of the bacterial nucleus was sharply defined. It was felt that these structures constitute true nuclei, and that there is a resting stage between each division, since the nucleus rounds up between the divisional stages. They likewise saw structures which strongly suggested a "mitotic-type figure," and they consider that each nucleus contains at least four chromosomes in *B. anthracis*. The preliminary observations of the author with phase contrast optics would tend to concur in general with the observations of Clifton & Ehrhard.

A Consideration of Mitosis in the Bacterial Nucleus

Evidence against mitosis.—Mitosis as a divisional mechanism of the bacterial nucleus has been described and documented by DeLamater (8, 9, 10, 36, 39, 40, 88 to 92). Before taking up the details of DeLamater's work, it seems proper to consider Bisset's extensive criticisms. Since they are the only extensively published specific criticisms of DeLamater's work on mitosis in bacteria which have been presented, and since they constitute a considerable volume of the literature in the field during the course of the last three years, it is felt justified to analyze his work in some detail.

Bisset has, on thirteen occasions, invoked the multicellularity of bacilli (94) as the basis for his criticisms of DeLamater's work, and has inferred, if not stated, that DeLamater believes all bacteria to be unicellular and multinucleate, despite photographic evidence and published statements to the contrary (9, 36) (see also Plate I, Figures 1 to 4, 5 to 8).

Bisset has variously considered "centrioles" in bacteria as derived in the

following ways: (a) as "artifacts derived from cross walls and septa" (47, 48); (b) by Hale's method, 1953, "granules at the junction of cell wall and cross wall" (34, 50); (c) "granular pools of stain at the junction of cell wall and cross wall" (52); (d) "derived from protoplasmic septa" (52).

"Centrioles" in *E. coli* are "provided by the material which in a non-septate bacterium corresponds to the septum in bacillus" or, otherwise stated, "are the basophilic areas at the points of division and growing tips of the cell" (50).

"Centrioles" in *Caryophanon* are "stainable areas which appear at or near the junction of cell walls and cross walls," and yet in the same paper it is stated, "DeLamater's mitotic figures occupy the space of two, three or four tiny discoid cells" (51), in which case there should be at least six, eight or ten "centrioles," respectively. It is obvious from the above that if a centriole is, or is derived from, one thing (i.e., "cross walls and septa"), it cannot be, or be derived from, another (i.e., "granular pools of stain"). Such interpretations of centriolar structures are mutually exclusive.

"Mitotic spindles" in bacilli are variously described by this same author as: (a) "basophilic growing points and septa" (47, 94); (b) "stained artifacts produced by method" (48); (c) "septa and cross walls" (48); (d) "concentration of stainable nucleic acids associated with the secretion and growth of the cross wall ('fragmented' chromosomes in natural positions)" (33); (e) "spindle is membrane material" (33); (f) in addition to calling the whole cell a nucleus, he described the "cell envelopes as portions of a mitotic spindle" (53). Again, Bisset's explanations of mitotic configurations are both inconsistent and mutually exclusive, since if a structure is one thing, such as a "stained artifact," it cannot, at the same time, be a "septum" or "cross wall," which are implied to be normal structures.

Whole nuclei (of DeLamater) have been variously described by Bisset as: (a) "a complete cell of this multicellular bacillus" (52); (b) "interphase, prophase and telophase nuclei were entire shrunken cells" (52); (c) "entire stainable contents of the cell may be mistaken for a nucleus . . . when methods of dehydration or fixation . . . cause unsuspected shrinkage" (53); (d) "cell membrane elements . . . repeatedly described as nuclei" (53); (e) "Structures associated with cell envelopes" are "nuclei" (53).

Here again the diverse structures described as representing the nucleus are inconsistent and mutually exclusive, since, if the entire contents of a cell "constitute a nucleus," then the "cell membrane elements" alone cannot simultaneously be the same structure. In addition, according to Bisset, DeLamater has been aided and abetted by "optical illusions" on at least two occasions (33, 94).

The above criticisms are directed at the work done on bacilli. In the coccus the situation is similar. Bisset has described "centrioles" in the coccus as: (a) "the nuclear bodies which occupy the centers of cells" (33); (b) "shrunken nuclei of these cells" (50); (c) "structures associated with cell envelopes" (53); (d) "granules at the junction of the cell wall and cross wall" (34).

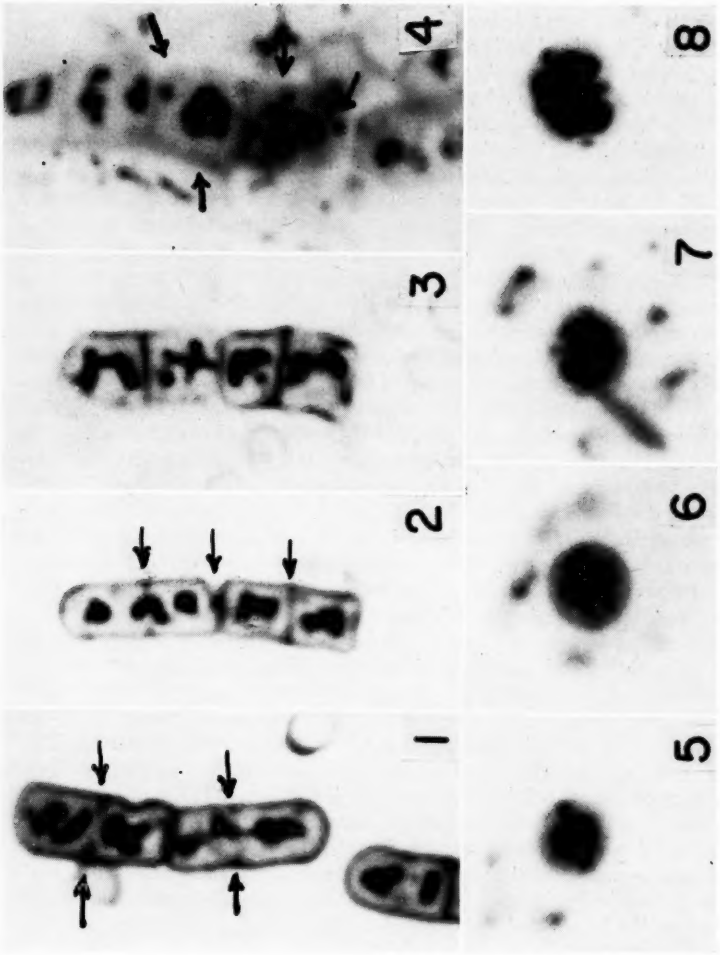


PLATE I

In this instance Bisset switches the basis of his criticism from a designation of the "shrunken nuclei" as centrioles to "structures associated with cell envelopes" or "granules at the junction of the cell wall or cross wall." In making this change he states that the "realization of the incorrectness of the basis of (his) first criticism underlines the incredibility of DeLamater's interpretation and brings the explanation of the appearances in cocci and bacilli more into line."

It would appear that Bisset's "explanations" are more important than the foundations upon which they are based, especially since parallel preparations rather than simultaneous differential stains on the same cell are used as the basis of his argument. In Bisset's recent article (34) on *Micrococcus cryophilus* he shows two-, three-, and four-celled cocci by Hale's method (135) and states that the cell membranes represent the mitotic configurations described by the author. He uses no double differential stains simultaneously showing the septa and nuclei. Further, he does not state the conditions of growth of his cells. Hunter & DeLamater (136), using both the tannic acid method and Hale's method, have found that under normal conditions (40)

←

FIGURES 1 TO 8
LEGENDS

Photographs 1 to 4 previously published (9, 36).

Photographs 5 to 8 by courtesy of Dr. M. E. Hunter.

Figures 1 to 4 *Bacillus megaterium*; Figures 5 to 8 *Micrococcus cryophilus* (first meiotic division).

All cells stained differentially for both nuclei and walls. Walls stained red; nuclei stained purple. ×4850. Figure 4 cell partially crushed. Figures 1 to 3 cells uncrushed.

FIG. 1. Lower rod bicellular. Upper rod bicellular. Lower cell shows early origin of cross wall from inner surface of wall or membrane. Upper cell shows later stage in wall development. Wall clearly perforated. This state most usual. Two upper nuclei in anaphase.

FIG. 2. Rod showing partial separation of cells by splitting of cross wall. Upper cell shows chromatinic material lying in perforation of cross wall. Cross wall of lower cell not yet complete; appears thin in center.

FIG. 3. Four cells in rod completely separated by cross walls. State usual in older cultures. Second lower nucleus in prometaphase with centriole distinct from wall.

FIG. 4. Walls stained red; centrioles and chromosomes, purple. Nucleus in anaphase. Lateral granule reddish purple, and interpreted as a mitochondrion.

FIG. 5. Metaphase spindle; wall red; two centrioles in polar positions seen as distinct granules separate from wall. Chromosomes on metaphase plate, blue.

FIG. 6. Early anaphase; walls red. Two centrioles at polar positions; two pairs of chromosomes just separating.

FIG. 7. Early telophase; walls red. Centrioles persist. Two nuclei separated by wall.

FIG. 8. Later telophase; walls red. Chromosomes elongating; centrioles persist; cross wall formed. Constriction of wall shows early stage of separation of cells.

only uni- and bicellular cocci are found. The bicellular state is obviously a division stage and is completely compatible with the nuclear picture described by DeLamater & Woodburn (40). Tetrads arising from large diploid cells by meiosis (81) are found under normal conditions in variable numbers. Whether these groups of four cocci are related to what Bisset calls four-celled cocci is unknown. Double differential stains, using the Hunter-DeLamater modification of Cassel's procedure, clearly separate the walls from the nuclear structures in diploid cells (81), although this procedure cannot be used on haploid cells with the same efficacy because of the low basophilia of the walls.

In cocci Bradfield (143) demonstrates the nucleus as a spherical central area which "contains bodies remarkably similar in form and arrangement to chromosomes." In Bradfield's Figure 2 (143) the chromosomes appear as paired threads suggesting identity with the chromatids of DeLamater (9, 36). He likewise indicates what he considers may be the nucleolus [see DeLamater & Woodburn (40)]. He believes all of the DNA[2] to be in the "central bodies" and, like Chapman & Hillier (75) and Birch-Andersen, Maaløe & Sjöstrand (76), could demonstrate no nuclear membrane. He demonstrates no cross walls or septa in a single round coccal cell in opposition to the claims of Bisset (34).

To further emphasize the difficulty in understanding Bisset's criticisms, he has variously described mitochondria in an entirely comparable manner (28) (see Mudd, Part I, this review). Here again, not only is the same structure variously interpreted (four different explanations in the same publication), but the same critically intended descriptions are used that are applied to the criticisms of nuclei and associated nuclear structures.

Others, also, have expressed doubt or criticism of the evidence of mitosis in bacteria. Robinow (95) has invoked the idea, based upon the earlier studies of Delaporte (96), that aggregates of fatty material press against the nuclear apparatus and distort it, producing the configurations described by DeLamater. Robinow bases his premise on the notion that DeLamater used old cells as his inocula (95). Here, as with Bisset's work, simultaneous nuclear and fat stains were not used. Large amounts of fatty substances occur in *Ascaris* eggs, for example, and do not appear to produce distortion of the nuclear apparatus. Robinow does not produce proof of such a distortion in bacteria. Further, he has stated (see above) that the nuclear structures described by DeLamater are present in his own water-mount preparations in both young cultures grown from spores and those initiated with young inocula.

A critical opinion of the concept of mitosis in bacteria has been expressed by Lamanna & Mallette (97) with no supporting evidence. These authors consider that DeLamater's interpretations are both premature and too extensive, and feel that his interpretation relies too heavily upon analogy to higher forms. In reply to this, it may be pointed out that the use of established terms to describe formations which appear to have the same structure and function as comparable elements in larger cells, can scarcely be con-

demned on the basis of analogy. Such usage of established terminology tends to prevent confusion caused by the application of a new and unestablished nomenclature. This resistance to the concept of mitosis in bacteria is readily understood. It rests on seventy years of unfounded precedent, and is abetted by the size of the cells under consideration. But neither precedent nor size are adequate reasons in view of the present evidence for maintaining this stand.

The evidence of Webb & Clark (131), presented in support of the concept of mitosis and even meiosis in *Micrococcus*, is not yet sufficient to be conclusive. The work of Chance (31, 31a) at this stage of its development, neither confirms, extends, nor refutes the original observations of DeLamater & Woodburn (40). The earlier work of Chance (137) has been cited previously (8) as constituting the first definitive evidence in support of a mitotic mechanism in bacteria. In opposition to precedent, Braun (138) and Goldschmidt (139) appear to have accepted the evidence favoring mitosis in bacteria as presented, or, as have Lederberg & Tatum (134), to have recognized the difficulties of both technical and interpretive nature inherent in the cytological problem, and look to the future for clarification. These geneticists have difficulty in imagining "how bacteria could get along without some sort of mitotic process, but its details require critical and objective definition." They also state that

> although nuclear aggregates that are very suggestive of mitotic metaphases and anaphases can be found with brief search, definitive interpretation of *E. coli* cytology depends for the most part on the validity of the conclusions that have been drawn from technically superior material.

Bradfield (143) believes the bacterial nucleus to be "moderately well defined" but that it "however, divides amitotically." Since he is dealing with sections and thus can visualize only a portion of the nuclear structure, he may not have seen the full structure. He is aware that a definitive statement cannot yet be made.

Evidence for mitosis.—The evidence for mitosis presented by DeLamater (8, 9, 10, 36, 39, 40, 88 to 92) is based upon a large volume of observations derived from several species of bacteria. Observations for the most part have been made on preparations stained by DeLamater's use of Östergren's (43) modification of the Feulgen procedure, and the freezing-dehydration technique developed first by Blank, McCarthy & DeLamater (46) for animal tissues, and subsequently applied to bacteria and other microorganisms by the author (39). Plate II summarizes schematically the concept of the bacterial nucleus, as summarized in the following pages. The reader is referred to the original papers (especially 8, 9, 36) for the photographic evidence.

Since the most extensive studies have been done on *B. megaterium*, the discussion will center mainly about this organism. In the normal cell during the logarithmic phase of growth, the nuclei have been observed to undergo

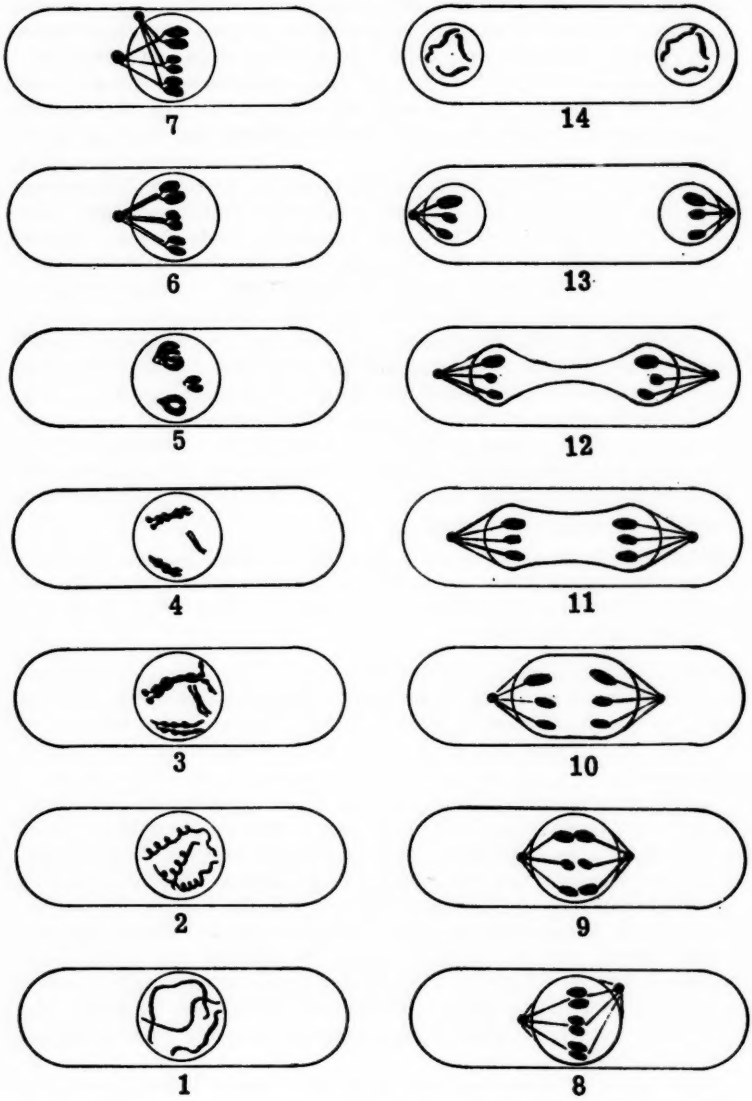


PLATE II

the following series of events (8, 9, 10, 36, 98). The chromosomes during interphase (as a starting point) lie in the nucleus as a tangle of long, delicate, beaded threads, which tend to lie adjacent to a nuclear membrane which can be visualized. During prophase the chromosomes contract progressively so that the beading, which appears to correspond to chromomeres of larger chromosomes, is readily observed. At about mid-prophase, when the chromosomes have contracted to definite rod-shaped structures, the longitudinal split of the chromosome into the two chromatids has been visualized. The three chromosomes can be counted and characterized at this stage. In the long chromosome of the haploid set of three there is a short central zone which appears common to both chromatids, and which is interpreted as the centromere. On the middle-sized chromosome the centromere appears to be subterminal or terminal. The centromere in the short chromosome has not yet been seen. Following the longitudinal split to form the chromatids, the chromosomes further contract; the identity of the chromatids becomes less obvious, but the chromosomes become more ribbon-like, and the corresponding or paired chromomeres on the chromatids can be readily seen. Subsequent to this, the chromatids appear to separate by the formation of loop-like configurations, and at prometaphase appear as paired granules attached to a single common point, suggesting that the chromatids, at prometaphase and after they line up on the metaphase plate, are held together by a centromere. This description is based upon studies presented in *International Reviews of*

←

PLATE II

FIGURES 1 TO 14

LEGENDS

Schematic presentation of author's concept of the bacterial nucleus and its divisional mechanism.

FIG. 1. Interphase: chromosomes long tangled threads.

FIG. 2. Early prophase: chromosomes contracting by spiralization.

FIG. 3. Middle prophase: chromosomes show longitudinal split forming chromatids. Chromomeres on two chromatids paired.

FIG. 4. Later prophase: chromosomes show further contractions. Chromatids still evident.

FIG. 5. Late prophase: chromatids separating.

FIG. 6. Prometaphase: chromosomes appear double as they line up on metaphase plate. Single centriole and spindle appear. Centriole extranuclear.

FIGURES 7 and 8. Prometaphase: two stages in migration of two centrioles (formed by division of one original) to poles. Spindle attached to nuclear membrane (?)

FIGURES 9, 10, and 11. Early, middle, and late anaphase: chromosomes pulled apart toward centrioles. Nuclear membrane pulled out.

FIG. 12. Late anaphase: nuclear membrane much elongated.

FIG. 13. Early telophase: nuclear membrane pulled apart, separating daughter nuclei. Centrioles and spindles persist.

FIG. 14. Late telophase: centrioles and spindles disappear. Chromosomes re-elongate.

Cytology (9) and the Rome *Symposium on Bacterial Cytology* (36), in which this sequence of events has been especially well documented. A full formulation of the author's concept of the bacterial nucleus, based upon all work done during the past four years, is to appear elsewhere with complete photographic documentation.

In early prophase, minute granules at the periphery of the nuclear vesicle, which appear to lie outside of the nuclear membrane, are to be seen. These have been interpreted as centrioles, and they have been observed to lie adjacent to, or close to the nuclear membrane. They then divide, and the two parts migrate to opposite sides of the nucleus. At metaphase there is a centriole at each pole of the nucleus with the chromosomes arranged on the metaphase plate between them. The metaphase stage appears to be very brief, and the chromosomes which form the metaphase plate separate in anaphase following, so far as can be made out, division of the centromeres of each chromosome. The anaphase stages are characteristic and are thought to produce the V- and U-shaped configurations which have been described particularly well by Robinow (7). In telophase the chromosomes are observed to re-elongate into the filamentous form, and the centrioles disappear. In many stages, it is evident, the chromosomal mass is too complex to be comprised of but one chromosome.

Growth of the organism in the presence of terramycin, streptomycin, colchicine and Na para-aminosalicylate has permitted a further analysis of nuclear activity (36, 98, 99, 100). Terramycin and aureomycin act similarly to produce mitotic arrest between prometaphase and early anaphase. They likewise cause a relatively tremendous enlargement of the centriole, and appear to affect the tenacity of the nuclear membrane. The migration of the much enlarged centrioles is arrested so that the stages in migration to the poles are clearly visualized. Observations indicate that at first the centriole is associated closely with the nuclear membrane, and subsequently migrates away from it. The association with the nuclear membrane is further accentuated by the action of Na para-aminosalicylate, it then being possible to define the direct attachment between the centriole and the membrane. Such configurations preclude Bisset's explanations. This drug appears to exert its effect by the suppression of centriolar division so that unipolar configurations are produced with single or doubled centrioles attached to one side of the nuclear membrane. The nuclear membrane here also appears to be thickened.

With the continued action of these drugs, it has been found that the nuclei continue to enlarge, and that the number of chromosomes within each multiplies so that what appears to be a polyploid condition is produced. The polyploid state seems to be induced at least in part by the thickened membrane of the nucleus, which interferes with the mitotic apparatus and prevents the separation of the chromosomes. When the action of the drug is decreased, either by dilution or degradation, the cells recover with pulling out and saculation of the nuclear membrane, each sac containing the vegeta-

tive haploid chromosome number. These observations are cited here to emphasize the continuity and persistence of the nuclear membrane (36, 98). The recovery phenomena, in which the haploid complements are segregated, suggest an association of the component chromosomes of each haploid set in the polyploid nucleus. The significance of this has yet to be ascertained, but it appears significant in the light of the newer genetic evidence (108, 109, 110).

The observations suggest that the mitotic process is essentially intranuclear, that the centrioles are cytoplasmic elements (98, 101) and are attached both to the nuclear membrane and to the chromosomes by means of the spindle element, which penetrates the nuclear membrane. They likewise appear to be capable of independent multiplication during the period of arrest and polyploidization (101).

In untreated cells the spindle element can be visualized as a definitive cone-shaped structure going from the centrioles to the individual chromosomes. In some of the photographs direct fibrous continuity between the centrioles and individual chromosomes has been visualized. In terramycin-treated cells, the spindle element may appear as a cone-shaped, clear space between the centriole and the aggregated chromosomal mass.

Marshak (114) has made the first effort at analysis of individual chromosomes with electron microscopy, with inconclusive results. The very beautiful electron microscope photographs of Chapman & Hillier (75), in which in their thin sections what are thought to be the nuclear structures are visualized as a central lighter core which appears to be pulled out with denser granules contained within it. The configurations suggest to the present author that the membrane of the bacterial nucleus remains intact during the nuclear division. Chapman & Hillier emphasize that they cannot visualize the nuclear membrane as a definite entity in their photographs. It appears likely that the processing of the preparations may have removed essential parts. The work of Birch-Andersen, Maaløe & Sjöstrand (76), in which the bacterial nucleus is recognized in their preparations as a sharply delineated vesicle with an aggregation of what is thought to be chromosomal material in the center, suggests also that the nuclear limits of the bacterial nucleus are clearly defined and that the chromosomes in their case have shrunken away from the membrane. The effects of processing, as in Chapman & Hillier's studies, may have removed parts of the membrane, rendering it less visible.

In "paracolon" Bradfield (143) apparently considers the cell has but a single nucleus. This is not in accord with the observations of Robinow (7), Kellenberger (22), DeLamater (9, 36), and many others, and may explain the projections of protoplasm which distort the nucleus in his thin sections. In Bradfield's Figure 7 is seen a large spherical nucleus of an actively growing cell in which the chromosomes appear as a tangle of paired threads; again, these are very suggestive of the chromatids of DeLamater (9, 36). Bradfield

states that the chromosomes are compact and well-defined only in dividing nuclei.

Obviously methods and the effects of processing are just now in the course of development and evaluation, but it would appear that an excellent groundwork is being laid. The unpublished phase contrast microscope observations of DeLamater further suggest that the membrane of the bacterial nucleus is pulled out during the nuclear division, and that the process is essentially intranuclear. Photographs presented by Robinow in several of his papers (7, 15, 16) and Knöll & Zapf (102) likewise indicate a continuity between these separating nuclear bodies, and it has been suggested (36) that the Giemsa stain in the Robinow procedure tends to adsorb to and, therefore, define the nuclear membrane in addition to the chromosomes. The very interesting and crucial work by Murray (32), in which he has studied the effects of fixation on the nuclear configurations of the bacterial cell, again suggest to the author of this review (36) that by means of fixation the bacterial membrane may be accented or minimized. Murray's figures likewise suggest that the membrane remains intact during the course of the mitotic division. The excellent phase contrast microscope studies of Clifton & Ehrhard (84) likewise lend support to this view. In this latter work, which deserves special emphasis, the bacterial nucleus is defined as a sphere in its interphase stage; the chromatinic bodies are demonstrated to consist of two, three, or six or more strands, which the authors feel may represent the chromosomes. Clifton & Ehrhard (84) indicate that their observations support the view that the nucleus is enclosed in a membrane. They likewise have observed "mitotic-type figures."

It would appear that the pertinent observations on the subject to date are tentatively interpretable as mutually supportive. As a working hypothesis or conceptual approach supported by extensive observations, the presence of a mitotic mechanism in bacteria has the advantage of being amenable to study from several points of view, including effect of temperature, drugs, proven mitotic inhibitors, ultraviolet light, x-ray, aging, and study by means of removal of specific cellular components with specific enzymes.

In the gram negative bacteria, *E. coli* (90) and *Caryophanon* (89), evidence for a similar mitotic process has been observed. In *E. coli*, which has been more completely studied, the process of nuclear division appears essentially similar to that already described for *B. megaterium*. The haploid chromosome number here likewise appears to be at least three (90).

Recent studies by DeLamater (36) and by Minsavage & DeLamater (103) on the action of colchicine on the gram negative *Salmonella typhosa*, have produced evidence entirely comparable to that described for the action of terramycin on *B. megaterium*. Since colchicine is thought to be a specific mitotic inhibitor, nuclear inhibition by this drug is considered to be strong supportive evidence for a mitotic process (123, 124). In *B. megaterium*, on the basis of chromosome counts, colchicine has been demonstrated to pro-

duce diploidization of the affected cells (36), which constitute between 5 and 10 per cent of the cell population.

Clifton & Ehrhard (84) have emphasized that they feel on the basis of their phase microscope observations that the so-called resting nucleus occurs between each nuclear division in the bacterial cell. This is in complete accord with the observations of DeLamater in terms both of the occurrence of a mitotic process and the contraction and elongation of the chromosomes which he has observed to occur in it.

Bisset (6) has described not only the vesiculate nucleus of bacteria as a specific entity [as have others (127)], giving to it a special significance which will be described in a moment, but has described likewise "primary vegetative nucleus," "spherical vegetative nucleus," "secondary nucleus," "rod-like nucleus," and the "primary nucleus." Exactly where each of these alleged types of nuclei fit into a consecutive divisional or growth process is not yet clear, if they do (4). This author, in addition to considering that "it is dubious whether the bacterial chromosome exactly corresponds to that of other cells" [see also Hoffman (23)], considers "the resemblance between the vesicular nucleus of *Azotobacter* and the nuclear vacuole of yeasts both in structure and behavior is very marked, and it is not unlikely that the nuclei of smaller bacteria, if fully resolved, would be found to share the resemblance." Since the nuclear structure in yeasts is still a very controversial subject (104, 105, 106, 130, 140), it is questionable whether this presumption of similarity is justified at this time. Bisset (6) likewise states

> that it must be concluded that the difference between the condition in bacteria which appear to possess a spherical vegetative nucleus and those in which the chromosomal condition is obvious and semi-permanent, lies in the power of the former to resume the resting form of the nucleus between each cell division, a state of affairs which is normal in most organisms.

It is questionable whether it is possible or justifiable to attempt to differentiate between the bacteria which have nuclear apparatus of the type described by Robinow and those with vesicular nuclei in the face of our present dearth of definitive knowledge, and in the presence of evidence suggesting that the two types represent stages in the same cycle (9, 36, 84).

Bisset (6) proceeds from a description of the vesiculate nucleus into a discussion of the "complex vegetative reproduction" in which he describes a fusion of nuclear components in the bacterial cell following the break-down of the transverse septa (133). There is then a segregation of units which he considers to constitute a reductional process. Many observers, including Robinow (7), Johnson & Gray (107), among others (8, 111, 132), have observed bacteria with these long central masses of chromatinic material. In the face of the genetic evidence produced by Lederberg (108), Cavalli (109), and Hayes (110), it seems that some other explanation should probably be sought. The work of Buzzati & Visconti (112) on x-ray hit curves suggests

that polyploid cells may occur in bacteria, and from the more recent work of DeLamater and his associates (36, 99, 100) on the effects of drugs in which polyploid nuclei have been demonstrated, it seems possible that this may form a working hypothesis for a solution of what these structures or cells are (see also Cassel, 125). This hypothesis has the advantage of being amenable to experimental analysis.

Cytologic Evidence for Sexuality in Bacteria

Sexuality in *E. coli* and *B. megaterium* has been evidenced by genetic studies (9, 108, 109, 109a, 110, 134). Evidence for a mechanism of cellular fusion by means of conjugation tubes has been presented by DeLamater (8, 141) and DeLamater & Hunter (142). This has been objected to by Bisset (33, 49, 50) with evidence that does not appear to be pertinent, as shown in Plate 8, Figure 11, Reference 8.

Hunter (81) has produced evidence demonstrating the homologous pairing of chromosomes in the "giant" cells of *Micrococcus cryophilus*, and the subsequent reductional or meiotic chromosomal configurations to be observed. The origin of the giant cells has not yet been followed.

Haploid and diploid cultures of *E. coli* K-12 have been studied cytologically (93, 134) and have revealed consistent differences, but doubling of chromosome number has not yet been unequivocally established. Preliminary studies of diploid cells of *B. megaterium* have suggested pairing of homologous chromosomes (9), but these studies, likewise, are not yet definitive. Further studies on genetically controlled cells of both *B. megaterium* and *M. cryophilus* are in progress.

The Nucleus of Spores

There are essentially two different schools of thought concerning the processes of sporulation and spore germination. Bisset (6), and Klieneberger-Nobel (111), particularly, among others (126), have described a nuclear fusion and reductional process as a part of sporulation. This follows the general outline of the work done by Schaudinn in 1902 and 1903 (116). The opposing school, represented currently by DeLamater & Hunter (117, 118) who studied *B. megaterium*, believes that the spores, at least in this organism, are laid down as a strictly vegetative reproductive process in the following way. Firstly, the last nuclear division prior to sporulation in the vegetative cell is a mitotic division of vegetative haploid nuclei, in which the haploid number can be seen. They could find no evidence for nuclear fusion prior to this division. Following this division, one nucleus remains condensed at one end of the now binuclear cell; the other expands to form a vegetative interphase nucleus in which the chromosomes are long, tenuous, tangled threads. This nucleus probably supports the metabolism of the bacterium during the process of spore formation. The spore is formed around and incorporates the condensed nucleus. When the spore is finally formed, the vegetative

nucleus coagulates and subsequently autolyzes. The spore may persist within the remaining bacterial membranes.

The first nuclear division on germination is mitotic, and the chromosome number in this nucleus likewise has been ascertained to be three, the haploid vegetative number. Subsequent divisions of the developing bacterium from this initial spore origin undergo characteristic mitotic processes. The recent observations of Robinow (119) on germinating spores, using DeLamater's procedures which he considers "delicate and very suitable," essentially confirm (Fig. 6) the nuclear processes of spore germination described by DeLamater & Hunter, although Robinow is not in full accord with the interpretation of mitosis given by these authors.

Robinow (120) originally considered that the spore nucleus was extracytoplasmic. Excellent, more recent studies by this same author (119) and by Fitz-James (121) have demonstrated clearly that the extracytoplasmic nucleus is an artifact produced by the hydrolyzing acid. He further indicates that once the spore has begun to germinate, the action of the acid will no longer produce the extracytoplasmic state of the nucleus. He considers that Hunter & DeLamater were describing germinating spores in all of their preparations, since they never observed the extracytoplasmic state. In more recent, as yet unpublished studies, Hunter & DeLamater have confirmed their previous observations and have found that the media on which the organisms are grown is important in defining whether or not the spores produced will render extracytoplasmic nuclei. In spores produced on nutrient agar, extracytoplasmic nuclei are consistently induced by HCl hydrolysis in the hands of these authors, whereas in spores produced on the medium used in their original studies the extracytoplasmic nuclei are seldom observed. It would appear that this phenomenon has not yet been fully clarified. It may be said that in other respects DeLamater and his collaborators and Robinow and his collaborators are in essential agreement not only on the problem of the spore nucleus, but likewise on the value of the thiazin dye-sulfurous acid mixtures as stains. Robinow, however, prefers water mounted cells for observation, while DeLamater prefers to use dehydrated mounts. Personal preference is the privilege of individual workers in such matters, so long as errors do not result.

Summary and Conclusion

A survey of the available evidence on the structure and divisional mechanism of the bacterial nucleus indicates, first, that the criticisms that have been made to date are not established on a firm foundation and do not refute the findings of DeLamater and his colleagues. This author's studies indicate that the bacterial nucleus is composed of discrete chromosomes which follow the pattern of chromosomal activity known for larger organisms, and possesses a mitotic apparatus consisting of a centriole and spindle. The whole mitotic process probably goes on as an intranuclear one within an intact

nuclear membrane, comparable to what is known to occur in many protozoa (122). This conceptual interpretation is posed as a working hypothesis amenable to further experimental approaches.

Chapman & Hillier, Birch-Andersen, Maaløe & Sjöstrand, Robinow & Fitz James, Murray & Wyckoff and, especially, Bradfield (143) have made measurable advances into the application of the electron microscope to bacterial cytology. Cassel, Hunter, and Hale have contributed to the knowledge of differential staining, while Murray has made a notable advance in our knowledge of the action of fixatives on the bacterial cell.

The question of polyploidy in bacteria has been raised by Buzzati & Visconti, and is being studied by means of specific mitotic inhibitors by Minsavage & DeLamater, while the antimitotic action of the antibiotics is being analyzed by Hunter, Szybalski, Bryson, and their colleagues.

Cytologic studies on the sexual phases of bacteria have just begun to be made. The ultimate pictures are yet to be drawn, and their correlations to the genetic mechanisms have yet to be made.

The next five-year period should prove fascinating with the fruition of studies currently in progress and the development of still newer methods and approaches to these interesting and difficult problems.

LITERATURE CITED

1. Bisset, K. A., *Ann. Rev. Microbiol.*, **5**, 1–16 (1951)
2. Lewis, I. M., *Bact. Revs.*, **5**, 181–230 (1941)
3. Knaysi, G., *Elements of Bacterial Cytology* (Comstock Publishing Co., Inc., Ithaca, N. Y., 375 pp., 1944)
4. Knaysi, G., *Elements of Bacterial Cytology*, 2nd ed. (Comstock Publishing Co., Inc., Ithaca, N. Y., 375 pp., 1951)
5. Piekarski, G., *Ergeb. Hyg. Bakteriol. Immunitätsforsch. u. Exptl. Therap.*, 26, 333–64 (1949)
6. Bisset, K. A., *The Cytology and Life History of Bacteria* (The Williams & Wilkins Company, Baltimore, Md., 136 pp., 1950)
7. Robinow, C. F., Addendum to R. J. Dubos' *The Bacterial Cell.*, 355–77 (Harvard University Press, Cambridge, Mass., 460 pp., 1949)
8. DeLamater, E. D., *Cold Spring Harbor Symposia Quant. Biol.*, **16**, 381–412 (1951)
9. DeLamater, E. D., *Intern. Rev. Cytol.*, **2**, 158–77 (1953)
10. DeLamater, E. D., Hunter, M. E., and Mudd, S. (Presented at Brookhaven Symposium on the Nucleus, Upton, N. Y., August, 1951); *Exptl. Cell Research* Suppl. II, 319–43 (1952)
11. Stille, B., *Arch. Mikrobiol.*, **8**, 125–48 (1937)
12. Piekarski, G., *Arch. Mikrobiol.*, **8**, 428–39 (1937)
13. Piekarski, G., *Zentr. Bakt. Parasitenk., Abt. I Orig.*, **142**, 69–79 (1938)
14. Piekarski, G., *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **144**, 140–48 (1939)
15. Robinow, C. F., *Proc. Roy. Soc.* (London), [B] **130**, 299–324 (1942)
16. Robinow, C. F., *J. Hyg.*, **43**, 413–23 (1944)
17. Klieneberger-Nobel, E., *Quart. J. Microscop. Sci.*, **91**, 340–47 (1950)
18. Tulasne, R., *Schweiz Z. allgem. Pathol. u. Bakt.*, **14**, 623–36 (1951)
19. Duguid, J. P., *J. Pathol. Bact.*, **50**, 265–74 (1948)
20. Flewett, T. H., *J. Gen. Microbiol.*, **2**, 325–33 (1948)
21. Kellenberger, E., *Experientia*, **8**, 99–105 (1952)
22[4]. Kellenberger, E., *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 45–66 (Charles C Thomas, Springfield, Illinois, 181 pp., 1953)
23. Hoffman, H. J., *J. Bact.*, **62**, 561–70 (1951)
24. Pringsheim, E. G., and Robinow, C. F., *J. Gen. Microbiol.*, **I**, 267–78 (1947)
25. Bringman, G., *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **156**, 547–56 (1951)
26. Knaysi, G., *J. Bact.*, **64**, 859–64 (1952)
27. Winkler, A., *Symposium: Bacterial Cytology*, supplement to *Rend. ist. super. sanità* 39–44 (Charles C Thomas, Springfield, Ill., 181 pp., 1953)
28. Mudd, S., in Part I of Mudd, S., and Delamater, E. D., *Ann. Rev. Microbiol.*, **8**, 1–22 (1954)
29. Welsch, M., and Nikoul, E., *Compt. rend. soc. biol.*, **142**, 1449 (1948)
30. Bielig, H-J., Kausche, G. A., and Haardick, H. Z., *Naturforsch.*, **46**, 80–91 (1949)
31. Chance, H. L., *J. Bact.*, **65**, 593–95 (1953)

[4] The Symposium on Bacterial Cytology (references 22, 27, 32, 35, 36, and 53) was published as a Supplement to the *Rendiconti dell' Instituto Superiore di Sanità* in Rome. The distributor for Europe and the British Empire, excepting Canada, is Blackwell Scientific Publications Ltd., Oxford, England; the distributor for the United States, Canada and South America is Charles C Thomas, Springfield, Illinois.

31a. Chance, H. L., *J. Bact.* **66**, 239–40 (1953)
32. Murray, R. G. E., *Symposium on Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 136–38 (Charles C Thomas, Springfield, Ill., 181 pp., 1953)
33. Bisset, K. A., *Cold Spring Harbor Symposia Quant. Biol.*, **16** (Discussion to DeLamater's paper) (1951)
34. Bisset, K. A., *J. Bact.*, **67**, 41–44 (1954)
35. Vendrely, R., *Symposium on Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 82–107 (Charles C Thomas, Springfield, Ill., 181 pp., 1953)
36. DeLamater, E. D., *Symposium on Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 108–35 (Charles C Thomas, Springfield, Ill., 181 pp., 1953)
37. Smith, A. G., *J. Bact.* **59**, 575–87 (1950)
38. DeLamater, E. D., *Stain Technol.*, **23**, 161–76 (1948)
39. DeLamater, E. D., *Stain Technol.*, **26**, 199–204 (1951)
40. DeLamater, E. D., and Woodburn, M., *J. Bact.*, **64**, 793–803 (1952)
41. Cassel, W. A., *J. Bact.*, **59**, 185–87 (1950)
42. di Steffano, H. S., *Stain Technol.*, **27**, 171–74 (1952)
43. Östergren, G., *Hereditas*, **34**, 510–11 (1948)
44. Atwood, K., and Ornstein, L., Amer. Assoc. Advance Sci. meetings. Abstract in program. (New York, N. Y., December, 1949)
45. Heubschman, C., *Mycologia*, **44**, 599–604 (1952)
46. Blank, H., McCarthy, P. L., and DeLamater, E. D., *Stain Technol.*, **26**, 193–97 (1951)
47. Bisset, K. A., *J. Gen. Microbiol.*, **8**, 50–56 (1953)
48. Bisset, K. A., *Stain Technol.*, **28**, 45–48 (1953)
49. Bisset, K. A., *Nature*, **167**, 247 (1952)
50. Bisset, K. A., *Science*, **116**, 154 (1952)
51. Bisset, K. A., *Mycologia*, **45**, 790–793 (1953)
52. Bisset, K. A., *Exptl. Cell Research*, **3**, 681–88 (1952)
53. Bisset, K. A., *Symposium on Bacterial Cytology*, supplement to *Rend. ist. super. sanità*, 9–23 (Charles C Thomas, Springfield, Ill., 181 pp., 1953)
54. Bell, L. G. E., *Intern. Rev. Cytol.*, **I**, 35–63 (1952)
55. Tulasne, R., and Vendrely, R., *Nature*, **160**, 225–26 (1947)
56. Leonardi, G., *Boll. soc. ital. biol. sper.*, **26**, 1–3 (1950)
57. Keigler, M. N., and Smith, A. G., *Cytologic Observations of the Effects of Enzymes on Acid-treated Spores of B. cereus*, *J. Hist. Cytochem.* (In press)
58. Hartman, P., and Payne, J. I., *Direct Staining of Two Types of Nucleo-proteins in E. coli*, *J. Bact.* (In press)
59. Tronnier, E. A., *Naturwissenschaften*, **40**, 511–12 (1953)
60. Chance, H. L., *Stain Technol.*, **27**, 253–258 (1952)
61. Quintini, J., and Tchan, Y. T., *Ann. Inst. Pasteur*, **77**, 188–92 (1949)
62. Cutinelli, C., and Lamanna, M., *Riv. ist. sieroterap. ital.* **25**, 1–8 (1950)
63. Cutinelli, C., *Nuovi ann. igiene e microbiolog.*, **I**, 208–16 (1950)
64. Califano, L., *Boll. ist. sieroterap. milan.*, **30**, 364–72 (1950)
65. Caselli, P., *Boll. soc. biol. sper.*, **23**, 1–3 (1947)
66. Welshimer, H. J., *J. Bact.*, **65**, 112–17 (1953)
67. Weibull, C., *J. Bact.*, **66**, 688–95, 696–702 (1953)
68. Kern, R. A., Kingkade, J., Kern, S. F., and Behrens, O. K., *J. Bact.*, **61**, 171–78 (1951)
69. Lembke, A., *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **156**, 377–80 (1951)

70. Mazia, D., *Sci. American*, **189**, 53–63 (1953)
71. Hillier, J., Knaysi, G., and Baker, R. F., *J. Bact.*, **56**, 569–76 (1948)
72. Hillier, J., Mudd, S., and Smith, A. G., *J. Bact.*, **57**, 319–38 (1949)
73. Mudd, S., and Smith, A. G., *J. Bact.*, **59**, 561–73 (1950)
74. Murray, R. G. E., and Wyckoff, R., *J. Bact.*, **66**, 242–44 (1953)
75. Chapman, G. B., and Hillier, J., *J. Bact.*, **66**, 362–73 (1953)
76. Birch-Andersen, A., Maaløe, O., and Sjöstrand, F. S., *Biochim. et Biophys. Acta*, **12**, 395–400 (1953)
77. DeLamater, E. D., Schaechter, M., and Hunter, M. E., *J. Hist. Cytochem.* (In press)
78. Robinow, C. F. (Personal communication, 1953)
79. Wenrich, D. H., (Personal communication concerning efficacy of method for protozoan *Trichomonas muris*, 1953)
80. Beutner, E. H., *J. Bact.*, **65**, 619–27 (1953)
81. Hunter, M. E., Abstract, *Proc. 9th Intern. Congr. Genet.* (Bellagio, Italy, August, 1953)
82. Robinow, C. F. (Personal communication, 1953)
83. Cassel, W. A., *J. Bact.*, **62**, 239–41 (1951)
84. Clifton, C. E., and Ehrhard, H.- B., *J. Bact.*, **53**, 537–43 (1953)
85. Tulasne, R., *Compt. rend. soc. biol.*, **143**, 1390, 1392 (1949)
86. Stempen, H., *J. Bact.*, **60**, 81–87 (1950)
87. Scanga, F., *Rend. ist. super. sanità*, **13**, 1–19 (1950)
88. DeLamater, E. D., *Bull. Torrey Botan. Club*, **79**, 1–5 (1952)
89. DeLamater, E. D., *Mycologia*, **44**, 203–6 (1952)
90. DeLamater, E. D., *Cold Spring Harbor Symposia* Quant. Biol., **18**, 99–100 (1953)
91. DeLamater, E. D., and Hunter, M. E., *Am. J. Botany*, **38**, 659–62 (1951)
92. DeLamater, E. D., and Mudd, S., *Exptl. Cell. Research*, **2**, 499–512 (1951)
93. Lederberg, J., Lederberg, E. M., Zinder, N. D., and Lively, E. R., *Cold Spring Harbor Symposia Quant. Biol.*, **16**, 413–43 (1951)
94. Bisset, K. A., *Intern. Rev. Cytol.*, **I**, 96–16 (1952)
95. Robinow, C. F., and Hannay, C. L., *Riassunti Comunicazioni, 6th Intern. Congr. Microbiol.*, **1**, **76** (Rome, Italy, 1953)
96. Delaporte, B., *Advances in Genet.*, **3**, 1–32 (1950)
97. Lamanna, C., and Mallette, M. F., *Basic Bacteriology* (Williams & Wilkins Co., Baltimore, Md., 677 pp., 1953)
98. DeLamater, E. D., *A Conceptual Formulation of the Structure and Division of the Bacterial Nucleus* (In preparation)
99. DeLamater, E. D., Hunter, M. E., Szybalski, W., Minsavage, E. J., and Bryson, V., *Riassunti 'Comunicazioni, 6th Intern. Congr. Microbiol.*, **1**, 48 (Rome, Italy, 1953)
100. DeLamater, E. D., Hunter, M. E., Szybalski, W., Minsavage, E. J., and Bryson, V. (In preparation)
101. DeLamater, E. D., *Abstr. Proc. Histochem. Soc.* (1954)
102. Knöll, H., and Zapf, K., *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **157**, 389–406 (1951)
103. Minsavage, E. J., and DeLamater, E. D., *Bact. Proc.*, 89 (1954)
104. DeLamater, E. D., *J. Bact.*, **60**, 321–32 (1950)
105. DeLamater, E. D., Yaverbaum, S., and Schwartz, L., *Am. J. Botany*, **40**, 475–92 (1953)
106. Widra, A., and DeLamater, E. D., *Bact. Proc.*, 89 (1954)

107. Johnson, F. A., and Gray, D. H., *J. Bact.*, **58**, 675–88 (1949)
108. Lederberg, J., *Proc. Nat. Acad. Sci. U. S.*, **35**, 178–84 (1949)
109. Cavalli, L. L., *Boll. ist. sieroterap. milan.*, **29**, 1–9 (1950)
109a. Cavalli, L. L., Lederberg, J., and Lederberg, E. M., *J. Gen. Microbiol.*, **8**, 89–103 (1953)
110. Hayes, W., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 75–94 (1953)
111. Klieneberger-Nobel, E., *J. Gen. Microbiol.*, **1**, 22–31, 33–37 (1947)
112. Buzzati-Traverso, A., and Visconti di Modrone, N., *Nature*, **162**, 295 (1948)
113. Murray, R. G. E., and Robinow, C. F., *J. Bact.*, **63**, 298–302 (1952)
114. Marshak, A., *Proc. Natl. Acad. Sci., U. S.*, **37**, 38–41 (1951)
115. Petronelli, A., *Boll. ist. sieroterp. milan.*, **30**, 499–501 (1951)
116. Schaudinn, F., *Arch. Protistenk.*, **1**, 306–43 (1902)
117. DeLamater, E. D., and Hunter, M. E., *J. Bact.*, **63**, 13–21 (1952)
118. Hunter, M. E., and DeLamater, E. D., *J. Bact.*, **63**, 23–31 (1952)
119. Robinow, C. F., *J. Bact.*, **65**, 378–82 (1953)
120. Robinow, C. F., *J. Gen. Microbiol.*, **5**, 439–57 (1951)
121. Fitz-James, P. C., *J. Bact.*, **66**, 212–19 (1953)
122. Schussing, B., *Handbuch der Protophytenkunde*, Bd. 1 (Gustav Fischer, Jena, Germany, 636 pp., 1953)
123. Levan, A., *Cold Spring Harbor Symposia Quant. Biol.*, **16**, 409–10 (Discussion to DeLamater's paper); 233–43 (Own paper) (1951)
124. Levan, A., *Hereditas*, **33**, 457–514 (1947)
125. Cassel, W. A., *J. Bact.*, **62**, 514–15 (1951)
126. Rolly, H., *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **157**, 407–19 (1951)
127. Clark, J. B., and Webb, R. B., *J. Bact.*, **66**, 498–500 (1953)
128. Scaletti, J. V., Cleverdon, R. C., and Newcomer, E. H., *J. Bact.*, **64**, 183–86 (1952)
129. Clark, J. B., Galyen, L. I., and Webb, R. B., *Stain Technol.*, **28**, 313–17 (1953)
130. Hartman, P. E., and Liu, C., *J. Bact.*, **67**, 77–85 (1954)
131. Webb, R. B., and Clark, J. B., *J. Bact.*, **67**, 94–97 (1954)
132. Bergersen, F. J., *J. Gen. Microbiol.*, **9**, 26–29 (1953)
133. Bisset, K. A., *J. Gen. Microbiol.*, **2**, 248–51 (1948)
134. Lederberg, J., and Tatum, E. L., *Science*, **118**, 169–75 (1953)
135. Hale, C. M. F., *Lab. Practice*, **2**, 115–16 (1953)
136. Hunter, M. E., and DeLamater, E. D. (Unpublished observations)
137. Chance, H. L., *J. Bact.*, **35**, 247–50 (1938)
138. Braun, W., *Bacterial Genetics* (W. B. Saunders Co., Philadelphia, Penna., 238 pp. 1953)
139. Goldschmidt, R., *Understanding Heredity. An Introduction to Genetics* (John Wiley & Co., New York, N. Y., 420 pp., 1952)
140. Lindegren, C. C., *The Yeast Cell, its Genetics and Cytology* (Educational Publishers, St. Louis, Missouri, 307 pp., 1949)
141. DeLamater, E. D., *Bull. Torrey Botan. Club.*, **80**, 289–98 (1953)
142. DeLamater, E. D., and Hunter, M. E., *J. Bact.*, **65**, 739–43 (1953)
143. Bradfield, J. R. G., *Nature*, **173**, 184–86 (1954)

THE NUTRITION OF MICROORGANISMS[1,2]

By David Hendlin

Research Laboratories, Chemical Division, Merck and Co., Inc., Rahway, New Jersey

The major advances in microbial nutrition during the past year have been made in the area of growth factor interrelationships. Many of the studies extended and consolidated previous findings, and for this reason some of the past literature has been drawn upon to provide background. As is the case with all reviews, space limitations and special interests of the author make impossible an exhaustive presentation of the literature. Some attention has been given in this review to the influence of physical environment on growth factor requirements and to antibiotics as they relate to nutrition, since these areas have received relatively little consideration in the past. On the other hand, those areas which have been covered adequately in previous reviews (1, 2) and which showed only limited progress during the year have been omitted.

Physico-Chemical Factors

Little attention has been given in the past to the significance of physical environment on the nutrition of microorganisms. Sporadic reports have appeared in the literature during the past fifteen years implicating such factors as temperature, pH, oxidation-reduction (O/R) potential, and CO_2 tension as factors affecting microbial nutrition.

According to Borek & Waelsch (3) temperature greatly affects the nutrition of *Lactobacillus arabinosus*. A temperature differential of 2°C. determined the essentiality of phenylalanine. Optimal growth occurred at 35°C. but not 37°C. unless the medium was supplemented with phenylalanine. Hills & Spurr (4) noted a similar relationship with respect to the amino acid requirements in *Pasteurella pestis*. In these cases, the changes in nutrition brought about by temperature could not be explained on the basis of selection of mutants. In general, such studies indicate that increases in temperature usually result in a gain of nutritional requirements. Recently, however, Campbell & Williams (5) have noted that with thermophilic bacteria increased temperature can also effect a loss of a nutritional requirement. The facultative thermophiles, *Bacillus coagulans* and several strains of *B. stearothermophilus*, required various amino acids and nicotinic acid at 36°C. but grew

[1] The survey of the literature pertaining to this review was concluded in December, 1953.

[2] The following abbreviations have been used in this chapter: O/R (oxidation-reduction); PGA (pteroylglutamic acid); CF (citrovorum factor); PABA (*p*-aminobenzoic acid); PA (pantothenic acid); LBF (pantetheine); ATP (adenosinetriphosphate); MSO (methionine sulfoxide); RNA (ribonucleic acid).

well without such supplementation at 45° and 55°C. Other thermophiles, however, showed additional nutritional requirements as the incubation temperature was raised from 36° to 45° and 55°C.

Studies with *L. lactis* Dorner and *L. leichmannii* indicated that the requirement for vitamin B_{12} by these species is dependent upon the O/R^2 potential of the medium. Anaerobiosis completely eliminated the requirement for vitamin B_{12} (6, 7). More recently, it has been observed that the pyrimidine requirement of *L. lactis* Dorner (uracil, thymine, or cytosine) is also dependent upon the O/R potential of the medium (8). While under aerobic conditions an absolute requirement for a pyrimidine exists, no requirement is demonstrable under anaerobic conditions. The converse was observed earlier by Richardson (9) with respect to the uracil requirement of *Micrococcus pyogenes* var. *aureus*. Stone & Hoberman (10) observed that anaerobiosis had a marked effect on the utilization of proline peptides by a prolineless mutant of *Escherichia coli*. These investigators demonstrated that under aerobic conditions proline peptides were utilized more readily than proline, whereas anaerobically, proline and the peptides were equivalent.

In studies with *Sarcina lutea* Brown & Binkley (11) noted that pO_2 influenced the nutrition of the microorganism. Although nicotinic acid was essential and thiamine stimulatory for growth at reduced pO_2, under highly aerobic conditions thiamine was essential and nicotinic acid stimulatory.

Several reports in the literature indicate that CO_2 tension plays an important role in the nutritional requirements of microorganisms. The amino acid requirements of *L. arabinosus* and *Streptococcus faecalis* were shown to be directly related to CO_2 tension (12). Broquist & Snell (13) have shown also that a CO_2 atmosphere greatly reduces the biotin requirement of bacteria. More recent studies indicate that as little as 1 per cent of CO_2 in the incubation atmosphere had a sparing effect on the biotin requirement of *L. lactis* Dorner (14).

It is becoming increasingly evident that physical environment profoundly affects the nutrition of microorganisms. This aspect of microbial nutrition will no doubt receive greater emphasis in the coming years.

Minerals

Recently an organo-iron compound, coprogen, has been found to be essential for the growth of the fungus, *Pilobolus kleinii* (15, 16). Coprogen is synthesized by many species of bacteria and fungi (15). Several chemically distinct forms are produced, each equally active for the test organism. Coprogen does not appear to be a porphyrin (17) and thus differs from the cytochromes. Another iron-containing compound, ferrichrome, has been isolated from *Ustilago sphaerogena* (18). Ferrichrome differs chemically from coprogen but is active for *Pilobolus* (19). While removal of iron does not inactivate ferrichrome for *Pilobolus*, acid hydrolysis results in complete inactivation. In addition, ferrichrome appears to be highly active in replacing the unknown factor required by *Arthrobacter terregens* (20). Ferrichrome biosynthesis by *Ustilago* is related to Zn^{++} concentration, large quantities of the compound

being produced in a Zn^{++}-deficient medium (21). The occurrence of these organo-iron compounds in many microorganisms and their requirement by others indicate that they may be of general importance and may function as iron-transporting systems.

Webb (22) had previously demonstrated that magnesium deficiency results in long chain formation among spore-forming bacilli (Bacillaceae) but was unable to demonstrate a similar phenomenon among cocci (*Micrococcus*, *Sarcina*, *Streptococcus*, and *Neisseria*) (23). Such long chain formation was reversed readily by the addition of Mg^{++} to the medium. Recently, Rochford & Mandle (24) noted a similar phenomenon with the pneumococcus. In Mg^{++}-deficient media, *Diplococcus pneumoniae* grew in long chains and readily reverted to the diplococcus form upon the addition of Mg^{++} to the medium. Such reversion was specific for Mg^{++} and could not be accomplished by Ca^{++}, Fe^{++}, or Mn^{++}. Concentrations of Mg^{++} below 0.4 μg. per ml. supported growth, but chains developed unless the concentration was raised to 0.8 μg. per ml.

Sheffner & Grabow (25) have demonstrated that for *Saccharomyces cerevisiae* Mg^{++} can be replaced by glutamine, asparagine, or glycine amide. Apparently Mg^{++} is involved in the biosynthesis of amide compounds in *S. cerevisiae*.

Camien & Dunn (26) had demonstrated earlier an interrelationship between Ca^{++} and serine in the nutrition of *L. casei*. Recently Eades & Womack (27) have found Ca^{++} to be stimulatory to *L. casei* grown in an amino acid medium. Optimum growth was obtained with 50 μg. Ca^{++} per ml. Calcium is also essential for the growth of *D. pneumoniae* (24) and *Chlorella pyrenoidosa* (28). For *Chlorella*, the Ca^{++} requirement can be replaced by strontium (28). Strontium also has been found to be stimulatory to the growth of *Rhizopus nigricans* (29).

Molybdenum and copper were reported to be essential for the growth of *C. pyrenoidosa* (28). In addition, Mo^{++} was demonstrated to affect markedly nitrate reductase in *Neurospora crassa* and *Aspergillus niger* (30). The nitrate reductase activity of these fungi was directly related to the Mo^{++} supplementation of the medium. Molybdenum may function in this instance as a component of the enzyme system as it does in xanthine oxidase (30a).

Lithium has been demonstrated previously to be toxic to several lactic acid bacteria (31). Recently MacLeod (32) has shown that Li^{+} toxicity is a function of hydrogen ion concentration, and that its toxicity is reduced with decrease in pH of the medium.

Vitamins

Vitamin B_{12}.—Since the early observations on the vitamin B_{12} requirement of *Euglena gracilis* (33), extensive nutritional studies of algal flagellates and nonflagellates have been undertaken. Through such studies Provasoli & Pintner (34) demonstrated that a wide variety of algae require vitamin B_{12} for growth. The authors concluded that vitamin B_{12} and thiamine are nutritional requirements which characterize the Euglenophyta.

Whereas the lactic acid bacteria and vitamin B_{12}-dependent strains of *Escherichia coli* responded to substances other than vitamin B_{12} (desoxyribosides and methionine respectively), *Euglena gracilis* was apparently highly specific in its vitamin B_{12} requirement, desoxyribosides and methionine being inactive. Hendlin & Woodruff (35), however, indicated that *E. gracilis* responded to substances of microbial origin other than vitamin B_{12}. Later Robbins, Hervey & Stebbins (36, 37) showed that pseudovitamin B_{12} was as active as cyanocobalamin for *E. gracilis*. More recently, Mollin & Ross (38) noted that urine and plasma samples from both normal and pernicious anemia subjects contained *Euglena*-active substances which were considered not to be vitamin B_{12}. These findings indicate unequivocally the nonspecificity of vitamin B_{12} for *Euglena*.

Both fresh water and marine chrysomonads were found to have an absolute requirement for vitamin B_{12} (*Ochromonas*, *Poteriochromonas*, and *Synura*) (34, 39 to 42). These microorganisms were highly specific in their requirement, desoxyribosides, methionine, and pseudovitamin B_{12} being inactive (39, 40, 42). Although methionine did not replace vitamin B_{12} in the nutrition of these chrysomonads, it exerted a marked sparing effect on the vitamin requirement (39). Unlike higher animals, the chrysomonads do not respond to choline or betaine, in combination with homocysteine. The phagotrophic nature of these organisms may make them suitable assay tools for the determination of the vitamin B_{12} content of particulate matter without recourse to complex extraction procedures (41). Further and widespread experience with this protozoan assay will be required in order to elucidate the specificity of the assay for vitamin B_{12}.

Earlier it had been reported that the acid degradation product of vitamin B_{12}, 5,6-dimethylbenzimidazole and its precursor, 1,2-dimethyl 4,5-diaminobenzene, are without vitamin B_{12} activity for *L. lactis* and *L. leichmannii*, and are in fact toxic to these organisms (43). Recently Robbins, Hervey & Stebbins (37) reported these compounds to be nontoxic and to possess an extremely low order of activity for *E. gracilis*. However, studies by Hendlin (44) indicated that these compounds as well as L-ribityl analogue of the dimethyl diamine are toxic to *Euglena* at levels of 90 to 270 μg. per ml. and that the toxicity is reversed by vitamin B_{12} over a narrow range of concentration.

Oginsky & Smith (45) observed that the addition of vitamin B_{12} to resting cells of the vitamin B_{12}-dependent strain of *E. coli* stimulated the acetate oxidation system. Vitamin B_{12a} (hydroxocobalamin), benzimidazole, 2,5-dimethylbenzimidazole, 5,6-dimethylbenzimidazole, and α-ribazole were without stimulatory effect in this system. More than that, 5,6-dimethylbenzimidazole inhibited the stimulatory effect of cyanocobalamin and interfered with the uptake of radioactive vitamin by cells grown in vitamin B_{12}-supplemented media, but not with the uptake by cells grown with methionine. The inhibitory effect of 5,6-dimethylbenzimidazole noted on the growth of *L. lactis*, *L. leichmannii*, *Escherichia coli*, and *Euglena gracilis* might well

result from its interference with the adsorption (or absorption) of the vitamin by these microorganisms.

Corbett (46) in investigating the *L. leichmannii* (ATCC 4797) assay noted that the presence of NaCl (1.5 mg. per ml.) in the assay medium markedly affected the sensitivity of the assay. The requirement for vitamin B_{12} was increased sixteen- to fortyfold by the presence of NaCl. Later Bardos & Gordon (47) studying the vitamin B_{12} requirement of *L. leichmannii* 313 observed that slightly hypertonic concentrations of inorganic salts inhibited the growth of the test organism. The inhibition was readily reversed with an added excess of vitamin B_{12}.

Davis & Chow (48) had demonstrated previously that the adsorption of vitamin B_{12} by *L. leichmannii* could be greatly reduced by the addition of salt. In the light of these observations, the phenomenon observed by Bardos & Gordon (47) could be ascribed to the inhibition of vitamin B_{12} uptake. Gordon, Heenan & Bardos (49), however, in a later publication concluded that their data indicated that salts had a direct ionic effect on the apoenzyme with which vitamin B_{12} combined.

Recently Daniel & Gray (50) observed an inhibitory effect of Mo^{++} on the growth of *L. leichmannii* ATCC 4797 which was reversed competitively by vitamin B_{12}. It seems probable that the Na_2MoO_4 inhibition is nonspecific and similar to that observed with other inorganic salts (46, 47).

Two years ago Beiler, Moss & Martin (51) reported that peroxide oxidation of cyanocobalamin in the presence of strong acid resulted in an oxidation product which proved inhibitory to *L. leichmannii* ATCC 4797 and that such inhibition was reversed by vitamin B_{12}. Villela & Abreu (52) in a study of a similar oxidation product with *E. gracilis* noted the previously described inhibition but were unable to reverse it with excess vitamin B_{12}.

Recently Hendlin & Wall (53) reinvestigated the toxicity of vitamin B_{12} oxidation mixtures. Under their experimental conditions, the toxicity observed appeared to be attributable solely to the relatively high levels of NaCl present in the reaction mixture and not to an oxidation product of vitamin B_{12}. Such toxicity was effectively antagonized by high levels of vitamin B_{12}. The salt toxicity and its reversal by vitamin B_{12} in these studies are similar to the earlier reports of Corbett (46) and Bardos & Gordon (47).

Pteroylglutamic acid (folic acid).—Work on the ornithine decarboxylase system of a strain of *Lactobacillus* indicated that pteroylglutamic acid is involved in the biosynthesis of pyridoxal phosphate (54). Cells grown in PGA-deficient medium were unable to phosphorylate pyridoxal to codecarboxylase, even when PGA[2] was added during the activation phase. In addition, 7-methylPGA, a PGA antagonist, was shown to inhibit decarboxylase activity.

Dewey & Kidder (55) in studies on the PGA requirement of *Tetrahymena geleii* noted that while thymine could not replace PGA for growth, both it and its desoxyriboside spared PGA. Vitamin B_{12} also had a sparing effect on the PGA requirement of *T. geleii*.

Previous reports have indicated that *T. geleii* was capable of utilizing aminopterin, a PGA antagonist, for growth in lieu of PGA (2). Similarly it has been shown that amethopterin (4-amino, N^{10}-methylPGA) and other PGA antagonists are capable of supporting growth of an amethopterin resistant strain of *S. faecalis* in PGA-deficient media. These experiments were interpreted to mean that both *T. geleii* and *S. faecalis* (resistant strain) were capable of converting these antagonists to PGA or were utilizing them per se (2). Recent reports from several laboratories indicate that some of the PGA antagonists (aminopterin, amethopterin, methopterin) used in nutritional studies are contaminated with quantities of PGA sufficient to account for their earlier observed PGA activity for microorganisms (56 to 59). These studies not only clarify the surprising results observed earlier with PGA antagonists, but also point to the care which must be exercised in the interpretation of experimental data obtained with "antivitamins" with reference to their utilization by microorganisms.

In studying a series of PGA analogues for toxicity to *T. geleii*, Dewey & Kidder (58) noted that 3′,5′-dichloro, N^{10}-methylPGA was an effective inhibitor. Because *T. geleii* appears to be highly resistant to the well-known PGA antagonists, these authors conclude that the dichloro analogue of methopterin should be a potent inhibitor against microorganisms resistant to other PGA antagonists.

Foley (60, 61) and Foley & Watson (62) recently have reported more extensively on the inhibitory nature of 1,2-dihydro-*s*-triazines. The *s*-triazines have been observed previously to be potent antagonists of PGA and its biological equivalents (2). With *S. faecalis*, the inhibitory effect of these compounds could be reversed by dihydroPGA, N^{10}-formylPGA and citrovorum factor but not by PGA. Substitution of a higher molecular weight substituent in position 2 resulted in marked reduction of inhibitory activity (60).

A strain of *S. faecalis* resistant to one of the substituted *s*-triazines (4,6-diamino-1-(3′,4′-dichlorophenyl)-1,2dihydro-2,2′dimethyl *s*-triazine showed cross-resistance to a number of related aldehydes and ketones (62). This fact suggests that, in these instances, resistance is to the general *s*-triazine structure rather than to specific substituents on the ring. The resistant strain showed no cross-resistance to substituted 2,4-diaminopyrimidines, aminopterin, or amethopterin, indicating that the locus of action of *s*-triazines is distinct from those effected by the PGA antagonists.

The 2,4-diaminopyrimidines are also potent PGA antagonists (2). Studies with *S. faecalis* indicate that development of resistance to one of these substituted pyrimidines (2,4-diamino-5(3′,4′ dichlorophenyl)-6-methylpyrimidine) is accompanied by a loss of requirement for PGA (63). Diaminopyrimidine-resistant cultures were cross-resistant to amethopterin, and, conversely, amethopterin-resistant cultures were insensitive to the diaminopyrimidine, indicating a common mode of action. Both amethopterin- and diaminopyrimidine-resistant strains of *S. faecalis* are apparently more efficient

in transforming PGA to citrovorum factor than the parent strains (63, 64).

2-DeaminoPGA was devoid of PGA activity and actually inhibited *S. faecalis* and *L. casei* but not PGA-independent microorganisms (65, 66). The inhibitory effect of the analogue for the lactic acid bacteria is reversed in a competitive manner by PGA and noncompetitively by leucovorin. When thymine is substituted for PGA, 2-deaminoPGA is nontoxic to these organisms. The partial growth (50 per cent of maximal) of *L. casei* obtained with thymine can be increased to maximal by supplementation of the medium with PGA or 2-deaminoPGA (66). This apparent activity of deaminoPGA may be due to its contamination with traces of PGA, as recently demonstrated for other PGA antagonists.

Citrovorum factor (folinic acid, leucovorin).—Sauberlich & Baumann (67) had reported that *Leuconostoc citrovorum* 8081 would not grow in chemically defined media unless they were supplemented with an unidentified growth factor designated Citrovorum Factor. Recently, Felton & Niven (68) in studying the morphological and biochemical characteristics of this strain of *L. citrovorum* found that this organism belongs to the genus *Pediococcus*. From an examination of several other species of this genus, it appears that the requirement for CF[2] is a unique characteristic of this group of microorganisms.

It has been reported that an apparent synergism exists between thymidine and CF (69). A similar phenomenon has been observed with PGA and N^{10}-formylPGA (70). When either of these two compounds is added to media in subminimal quantities, the response of *L. citrovorum* to CF is increased three- to fourfold. Such synergism is probably explained by the fact that *L. citrovorum* is capable of synthesizing CF from PGA once growth is initiated (70).

Studies made on the biosynthesis of CF by microorganisms which require PABA,[2] rhizopterin, and PGA indicate that they all synthesize CF from an appropriate precursor (64, 70, 71). In all cases, the major part of CF activity was in a bound form available to *L. citrovorum* only after enzymatic digestion (70). Studies with resting cells indicated that reducing conditions were essential for high conversions. Sufficiently low O/R potentials could be obtained by the addition of ascorbic acid (64, 70), glucoascorbic acid, glutathione, thioglycolic, and thiomalic acids (64) or by incubating the reaction in an atmosphere of nitrogen (64). Reducing conditions were essential also in the chemical synthesis of the factor (72) as well as in the biosynthesis of CF by liver slices *in vitro* (73). In studies with *S. faecalis*, formate was also observed to be essential for optimal conversion of PGA and related compounds to CF (64). In this respect, serine replaced formate, but glycine, a known source of "active formate," had no effect. Rhizopterin, N^{10}-formylPGA, and tetrahydroPGA were also converted readily to CF by resting suspensions of *S. faecalis* (64, 70). Surprisingly, a C_1 compound and reducing conditions were also essential for maximal conversion of the formyl and tetrahydro derivatives of PGA (64).

Dietrich, Monson & Elvehjem (74) reported that in chicks vitamin B_{12} enhanced the conversion of PGA to CF. Such was not the case in studies with the lactic acid bacteria (70). With these microorganisms, vitamin B_{12} had no effect whatever on the biosynthesis of CF.

Aminopterin and x-methyl PGA have been found to inhibit completely the conversion of PGA to CF by resting cells of *L. casei*, *L. arabinosus*, and *S. faecalis* (70). These observations are similar to those reported by Nichol & Welch for rat liver slices (75). *L. arabinosus* was able to convert PABA to CF, a conversion which was inhibited by sulfanilamide as well as aminopterin (70). Wold & Sirny (76) noted that L-alanine inhibited the biosynthesis of serine by *L. mesenteroides* P-60. The inhibitory effect of L-alanine was reversed completely by 50 mμg. per ml. of CF but not by PGA.

Pantothenic acid.—Lansford & Shive (77) had demonstrated previously that for an *E. coli* mutant requiring pantothenic acid or pantoic acid, the α-ketolactone of pantoic acid possesses approximately three times the activity of pantoic acid. For this mutant, α-ketoisovaleric acid and its corresponding alcohol were devoid of activity. Recently Maas & Vogel (78) have corroborated the findings of Lansford & Shive and have extended the studies through the use of mutants of *E. coli* and *Aerobacter aerogenes* in which the biochemical block appeared earlier in the biosynthetic sequence. Mutants were obtained for which dihydroxyisovaleric, α-ketoisovaleric, and α-ketopantoic acids were active in supporting growth. These observations have permitted Maas & Vogel (78) to postulate the following biochemical sequence for pantoic acid synthesis.

$$(CH_3)_2COHCHOHCOOH \rightarrow (CH_3)_2CHCOCOOH \rightarrow$$
$$CH_2OHC(CH_3)_2COCOOH \rightarrow CH_2OHC(CH_3)_2CHOHCOOH.$$

Early experiments by King, Stearman & Cheldelin (79) implicated PABA in the biosynthesis of PA.[2] Pomper (80) later observed that PA was able to spare the PABA requirement of a mutant strain of *S. cerevisiae*. Recently it has been shown that *Bacterium linens* requires either PA or PABA for growth (81). These studies have been reviewed in detail elsewhere (2).

Purko, Nelson & Wood (82) have observed also an interrelationship between biotin and PA for *B. linens* since, under their conditions, biotin stimulated growth in a PA- or PABA-supplemented medium and reversed sulfanilamide toxicity in PABA-containing medium. An interrelationship between biotin and PA has been demonstrated also for a pantothenicless mutant of *Neurospora crassa* by Ryan, Kunin & Ballentine (83). Biotin was toxic to the mutant and the toxicity was reversed by PA. The inhibitory effect of biotin appears to be on the rate of utilization of PA by *N. crassa*. Further evidence for the interrelationship between biotin and PA is presented by Pittillo & Foster (84) who noted that isonicotinic acid hydrazide inhibition of *A. aerogenes* is reversed by PA or biotin.

Much has been learned of the mechanism whereby pantetheine arises from PA. Brown & Snell (85) using acetone powders of *Proteus morganii* got a product from interaction of cysteine, ATP[2] and PA which was more ac-

tive than PA for *Acetobacter suboxydans*. The reaction product was, however, inactive for *S. carlsbergensis*, *L. casei*, and *L. arabinosus*. The inability of thioethanolamine to substitute for cysteine, among other reasons, led the authors to conclude that the active product was N-pantothenylcysteine (PA-cysteine). PA-cysteine was approximately three times more active than the corresponding disulfide and sixteen times more active than PA in supporting growth of *A. suboxydans*. Dried cell preparations of *A. suboxydans* were apparently able to decarboxylate PA-cysteine to LBF[2] according to the following scheme (85):

$$\text{PA-cysteine} + \text{ATP} \rightarrow \text{phosphoPA-cysteine} \rightarrow \text{phosphoLBF} + CO_2.$$

It would appear that PA-cysteine and phosphoPA-cysteine as well as their disulfides may be several of a group of compounds which comprise *A. suboxydans* "stimulatory factor."

King & Cheldelin (86) in an attempt to further elucidate the nature of this stimulatory factor studied a series of PA derivatives for growth-promoting activity for *A. suboxydans*. Coenzyme A (CoA) and γ-phosphoLBF were both twenty times more active than PA for *A. suboxydans*. LBF and PA-cysteine (less active than LBF) were more active than PA. High activity appeared to be associated with the reduced forms of the compounds tested. Autoclaving of compounds with medium supplemented with cysteine always resulted in activities significantly higher than those obtained by aseptic addition of filtered solutions to the medium (86).

Vitamin B_6 group.—The biological activity of pyridoxylamines in the rat (87) prompted a study of these analogues in the nutrition of microorganisms. Rabinowitz & Snell (88) found that a series of pyridoxylamines had no significant vitamin B_6 activity for *Saccharomyces carlsbergensis*, *Streptococcus faecalis*, and *L. casei*. However, several of the analogues possessed activity for *Neurospora sitophila*. Such activity was taken to be indicative of enzymatic conversion of the pyridoxylamines to pyridoxamine or pyridoxal.

Previously reported to inhibit *S. cerevisiae* (89), 4-desoxypyridoxine was also toxic to *S. carlsbergensis* and *N. sitophila*, but not to the vitamin B_6-dependent lactic acid bacteria (90). The toxicity was antagonized competitively by pyridoxine and to a lesser extent by pyridoxal and pyridoxamine. When the sensitive organisms were cultured under conditions which allowed synthesis of vitamin B_6, 4-desoxypyridoxine had little, if any, effect (90).

The toxicity of the 5-desoxy analogues of vitamin B_6 for *S. carlsbergensis* was antagonized most effectively by pyridoxine (91). These analogues were less toxic than 4-desoxypyridoxine. In contrast to 4-desoxypyridoxine, the 5-desoxy compounds were inhibitory to the vitamin B_6-dependent lactic acid bacteria, their degree of effectiveness depending upon the particular organism used.

When *S. faecalis* was made independent of vitamin B_6 by culturing in D-alanine supplemented medium, the antagonists were no longer active (91). Rabinowitz & Snell (91) indicated that 4-desoxypyridoxine and the 5-desoxy analogues act at different loci, since their antagonistic effects are not addi-

tive. ω-Methylpyridoxine (substitution of an ethyl for a methyl group in position 2 of the pyridine nucleus) was also found to be a potent vitamin B_6 antagonist with activity similar to 4-desoxypyridoxine (91).

It has been shown that isoniazid (isonicotinic acid hydrazide) inhibits amino acid decarboxylase of *E. coli* (92) and thus may be functioning as an antimetabolite of the vitamin B_6 group (92, 93). Recently Boone & Woodward (94) studied the relationship between isoniazid and vitamin B_6 in microorganisms. These investigators observed that the toxicity of isoniazid for several microorganisms could be reversed competitively by vitamin B_6. For *L. arabinosus* pyridoxamine was the most effective reversing agent, while pyridoxal, pyridoxamine, and pyridoxine were equally effective for *S. carlsbergensis*. However, for several mycobacteria isoniazid inhibition was not reversed by the vitamin B_6 group. Reversal of isoniazid toxicity for *A. aerogenes* also was obtained with pyridoxamine (84).

Moulder & Woods (95) have reported recently that the inhibitory effects of nicotinic acid and adenine on the growth of *S. cerevisiae* can be antagonized equally well by all three members of the vitamin B_6 group.

Biotin.—Campbell & Williams (96) demonstrated that for a large number of facultative thermophilic bacteria biotin is an essential nutrient. For these microorganisms, oxybiotin possessed biotin activity irrespective of the incubation temperatures employed. Desthiobiotin showed similar activity except with one strain of *B. coagulans*. With this strain, desthiobiotin was effective in replacing biotin only at 36°C. but not at 45° or 55°C.

Pimelic, oleic, and aspartic acids, effective as biotin substitutes for other microorganisms (97, 98), were capable of supporting growth of several of these thermophiles but such substitution for biotin was wholly dependent upon temperature of incubation. These observations further emphasize the importance of physical environment on the nutritional requirements of microorganisms.

In their earlier report on the interrelationship of biotin and fatty acids, Broquist & Snell (13) concluded that, for several lactic acid bacteria under their experimental conditions, biotin and oleic acid were nutritionally equivalent. More recent results obtained by these investigators indicate that this is not the case and that oleic acid merely spares biotin (99). Biotin, but not oleic acid, supported growth of *L. arabinosus* in a medium supplemented with avidin. This observation and the demonstrated reversal of ureylenecyclohexylbutyric acid toxicity by biotin warrant the conclusion that biotin is essential for growth of *L. arabinosus* and that the requirement can not be wholly satisfied by fatty acids (99).

Recently Pittillo & Foster (84) showed that the antibacterial action of isoniazid for *A. aerogenes* was reversed by biotin. Isoniazid appeared to be inhibiting the biosynthesis of biotin by this microorganism.

Amino Acids and Peptides

Lampen, Jones & Perkins (100) observed that methionine sulfoxide, but not the sulfone, was equivalent to methionine in supporting the growth of a

methioneless strain of *E. coli*. The biological activity of MSO[2] was ascribed to the ability of *E. coli* to reduce it to methionine. More recently Sourkes & Trano (101) have shown that MSO is reduced by *E. coli* via a hydrogenase system, but that the organism is unable to reduce the sulfone. MSO also has been found to support full growth of methionineless strains of *S. cerevisiae* [Pomper (102)].

MSO has been shown to be toxic to *L. arabinosus*, the toxicity being competively antagonized by glutamic acid (103). MSO apparently inhibits synthesis of glutamine from glutamate [Waelsch *et al.* (103); Elliot & Gale (104)]. Recently Krask (105) demonstrated that *B. subtilis* sporulation, which is dependent upon glutamic acid, is inhibited by MSO. At a molar ratio (MSO:glutamate) of 1:7, growth was unaffected but sporulation was completely inhibited.

Kalan & Ceithaml (106) have obtained methionineless strains of *E. coli* capable of limited growth in the presence of homoserine, alanine, valine, isoleucine, and α-aminobutyric acid. With these substrates, growth could be enhanced by the addition of vitamin B_{12} to the medium. These results apparently support the view that vitamin B_{12} is involved in the biosynthesis of methionine (107) and that several amino acids are capable of supplying the C_4 skeleton of methionine (106).

Shapiro (108) has obtained methionine auxotrophs of *A. aerogenes* capable of growing on thiomethyladenosine or methionyladenosine. These auxotrophs, however, grow better on methionine and can not utilize homoserine, threonine, or α-aminobutyric acid, with or without vitamin B_{12} as replacements for methionine.

The biosynthesis of methionine in *Neurospora* (109), *E. coli* (110), and *B. subtilis* (111) has been established as occurring through the following reaction sequence:

cysteine→cystathionine→homocysteine→methionine.

Recently Pomper (102) in studies with methionineless strains of *S. cerevisiae* noted that cysteine but not cystathionine would support the growth of his strains. These results apparently speak against the above sequence in *Saccharomyces*. More recently Schwartz & Shapiro (112) using *A. aerogenes* auxotrophs which responded to methionine or thiomethyladenosine presented evidence in support of a new concept in methionine biosynthesis. These authors proposed on the basis of growth, enzyme, and radioactive studies, that methionine is synthesized by the direct transfer of the thiomethyl group of thiomethyladenosine to a C_4 compound, probably α-aminobutyric acid. While the data supporting such a hypothesis is strong, the activity of homocysteine for these mutants appears to be incompatible and requires clarification (112).

Cohn, Cohen & Monod (113) and Wijesundera & Woods (114) have demonstrated that the biosynthesis of methionine by *E. coli* and *A. aerogenes* is inhibited completely by the presence of methionine in the basal medium. Methionine at concentrations of 10^{-4} M inhibited biosynthesis, while cys-

teine, cystathionine, and homocysteine were without effect. *E. coli* recovered the ability to synthesize methionine when it was subcultured on a methionine-deficient medium (114).

The inhibitory effect of threonine for a strain of *N. crassa* appears to be on the methylation of homocysteine to methionine (115). In this instance choline partially counteracts threonine inhibition, presumably by acting as a methyl donor.

Acetylamino acids appear to be amino acid precursors for *E. coli*. Vogel (116) presented data showing that acetylglutamic acid and acetylornithine are intermediates in the biosynthesis of ornithine by *E. coli*.

Two general transaminase systems were described for *E. coli* by Rudman & Meister (117). Transaminase A was involved in the transamination of the aromatic amino acids (phenylalanine, tryptophan, and tyrosine) and transaminase B in the transamination of the aliphatic amino acids (leucine, isoleucine, valine, norvaline, and norleucine). The inability of an isoleucineless mutant (42–37) to utilize α-keto-β-methylvalerate was ascribed to lack of transaminase B. A second isoleucineless mutant (97–21) capable of growing on the ketomethylvalerate contained transaminase B. Both the mutant (97–21) and the wild type *E. coli* were able to use isoleucine as a sole nitrogen source for growth. Rudman & Meister (117) offer this observation as further evidence that utilization of amino N depends upon the ability of an organism to deaminate the amino acid via transamination.

The double requirement for isoleucine and valine in *E. coli* (117, 118) and *Neurospora* (119) was ascribed to the inhibition of transamination of "ketovaline" by "ketoisoleucine." Rudman & Meister (117) propose an alternative explanation for their double mutant *E. coli* strain, namely that this mutant lacks the transaminases for both valine and isoleucine. This is probably not the case for *Neurospora* since "ketovaline" is equally as effective as valine in supporting growth of the mutant [Bonner (119)]. In addition, in *Neurospora*, "ketoisoleucine" has been shown to inhibit the growth-promoting effect of "ketovaline" but not that of valine (119).

Umbarger (120) reported an *E. coli* mutant whose amino acid requirements appear to be related to the carbon source used. When glucose, glycerol, pyruvate, arabinose, or gluconic acid are added to an adequate medium (acetate-salts medium), optimal growth does not occur unless glutamic acid, aspartic acid, lysine, and proline are supplied.

Rowley (121) observed amino acid antagonisms among 350 strains of *E. coli*. Various amino acids were toxic to *E. coli* in an NH_3-N medium and the toxicity was neutralized by other amino acids. Norvaline and norleucine were inhibitory for the vast majority of strains studied, and in most cases methionine reversed the inhibition.

Previously α-methylglutamic acid was shown to inhibit glutamic acid utilization by *L. arabinosus* [Ayengar & Roberts (122)]. Recently Roberts & Hunter (123) found α-methylaspartic acid to be an effective competitive inhibitor of aspartate utilization by *L. mesenteroides* P-60. The hydrazide of

aspartic acid was also toxic but not the α-methyl analogue of asparagine.

Previously Shive & Macow (124) demonstrated that hydroxyaspartic acid was antagonistic to aspartate for *E. coli*. Recently Buston, Churchman & Bishop (125) have synthesized hydroxy analogues of valine and norvaline. Hydroxyvaline possessed no valine activity for *L. arabinosus* 17-5 and actually inhibited the growth of the organism. The hydroxynorvalines (isomeric homologues of threonine and allothreonine) could not support growth of *L. arabinosus* or *S. faecalis*. One of them (homologue of threonine) was antagonistic to the threonine requirement of *S. faecalis*.

Davis (126) in studying the biosynthesis of the aromatic ring in microorganisms had shown through the use of *E. coli* mutants that shikimic acid is the precursor of the amino acids, tryptophan, phenylalanine, and tyrosine. Extension of these earlier studies with mutant strains of *E. coli* in which the genetic blocks occurred prior to shikimic acid has permitted further elucidation of the sequence in the biosynthesis of the aromatic ring. Such mutant strains accumulate considerable quantities of precursor demonstrable in their culture filtrates which can be assayed with the appropriate mutant. By such techniques, Salamon & Davis (127) have been able to isolate and identify as the precursor of shikimic acid, 5-dehydroshikimic acid. Continuation of these studies by Weiss, Davis, & Mingioli (128) has indicated that 5-dehydroshikimic acid is derived from a substituted cyclohexane, 5-dehydroquinic acid.

Shiguera, Sprinson & Davis (129), using radioactive glucose to elucidate the mechanism of shikimic acid formation, noted that shikimic acid does not arise as believed earlier via cyclization of glucose but rather through the condensation of two fragments derived from glucose. Thomas and coworkers (130), in studying the biosynthesis of tyrosine by yeast from radioactive acetate and pyruvate, propose that the aromatic ring is formed through the condensation of two unsymmetrical C_4 acids. Such a hypothesis is in accord with the foregoing observations on the biosynthesis of shikimic acid from glucose in *E. coli*. The side chain of tyrosine was found to arise from pyruvate as an intact unit (130). This observation in yeast corroborates the earlier findings on the origin of the side chain of tyrosine in *E. coli* [Cutinelli *et al.* (131)].

From studies on the biosynthesis of the aromatic amino acids, Davis (126) concluded that these amino acids arise from a common precursor, shikimic acid, but by independent routes. Beerstecher & Shive (132), however, by the application of inhibition analysis had concluded that tyrosine arises via oxidation of phenylalanine. They based their conclusions on the fact that tyrosine is much more active than phenylalanine in reversing the toxicity of β-2-thienylalanine for *E. coli*. Simmonds, Tatum & Fruton (133), on the other hand, were unable to find any sparing effect of tyrosine on the phenylalanine requirement of a phenylalanineless mutant of *E. coli*. Recently, however, Bergmann, Sicher & Volcani (134) demonstrated that, with a phenylalanineless mutant of *E. coli*, tyrosine does spare phenylala-

nine. They ascribe the differences between their experiments and the earlier negative ones (133) to differences in technique. Although phenylalanine and tyrosine spared the trypotophan requirement of the tryptophanless strain, no sparing effect was observed with phenylalanine or tryptophan for the tyrosineless mutant. These findings in addition to their observations with various aromatic amino acid antagonists have prompted the authors to conclude that the biosynthetic pathway for the aromatic amino acids is:

precursor → tryptophan → phenylalanine → tyrosine.

The complexity of the formation of the aromatic amino acids probably can not be explained completely either by Davis' (126) independent route hypothesis or by the straight chain sequence postulated by Beerstecher & Shive (132) and Bergmann, Sicher & Volcani (134).

Many studies on the nutrition of bacteria have indicated a growth requirement for partial hydrolyzates of casein. Other studies have shown that crystalline peptides possess varying degrees of activity in promoting growth of microorganisms ranging from less active to more active than the corresponding amino acids. In most instances, peptides have been less or equally as active as growth-promotants as their constituent amino acids. Such utilization of peptides was said to be preceded by hydrolysis. In a smaller number of cases peptides were more effective in supporting growth than their related amino acids. Such enhanced activity has led to the conclusion that peptides are utilized *in toto*. Recently Kihara & Snell (135) and Kihara, Klatt & Snell (136) have indicated two basic reasons why peptides are more active in the nutrition of lactic acid bacteria. They observed that the inhibitory effect of D-alanine for *L. casei* is more readily reversed by L-alanyl peptides than by the amino acid, L-alanine. In the case of *S. faecalis*, the inability of codecarboxylase to degrade tyrosyl peptides as rapidly as it decarboxylates tyrosine may explain why tyrosyl peptides are more effective in supporting growth of the organism than the corresponding amino acid. More recently Prescott, Peters & Snell (137) have studied the activity of serine peptides. These peptides were found to be 15 to 90 times as active as L-serine in supporting the growth of *L. delbreuckii* 9649. In this instance, the high activity observed for serine peptides was attributable to the greater efficacy of such peptides in reversing the toxicity of D-alanine. The reversal by peptide and not by the amino acid was noncompetitive. The activity of serine peptides for *L. delbreuckii* is analogous to alanyl peptides for *L. casei* (135).

Stone & Hoberman (10) reinvestigated the high degree of activity of prolyl peptides for a prolineless mutant of *E. coli* earlier described by Simmonds & Fruton (138). They observed that while proline was rapidly deaminated by *E. coli* under the experimental conditions used, prolyl peptides were unaffected by proline deaminase. It was found that growth curves with prolyl peptides show an initial lag which is not observed with proline

[Stone & Hoberman (10)]. This fact indicated to the authors that the peptides undergo hydrolysis prior to utilization.

Hirsch & Cohen (139) observed that although leucyl peptides were no more active than leucine in supporting growth of a leucineless mutant of *E. coli*, the peptides were many times more effective than the amino acid in reversing the inhibitory effect of isoleucine. The reversal by peptides was noncompetitive and highly specific in that non-leucine-containing peptides had no effect whatever on isoleucine toxicity. The authors conclude that the peptide is probably utilized *in toto* since resting cell studies demonstrated no appreciable hydrolysis.

Histidine peptides were found to be more effective than histidine alone in supporting growth of *L. delbreuckii* [Peters, Prescott & Snell (140)].

The foregoing studies, especially those of Snell and his coworkers (135, 136, 137, 140), have done much to clarify the role of crystalline peptides in microbial nutrition and the apparent essentiality of enzymatic digests of proteins for many microorganisms. The explanations offered do not, however, necessarily elucidate the role of peptides in all instances. Although most of the available data indicate the occurrence of hydrolysis of the peptide bond, there is still some evidence indicating that hydrolysis does not occur. The fact that peptides are more effective than their amino acid counterparts in supporting growth of microorganisms does not necessarily preclude the hydrolysis hypothesis.

In several instances, polypeptides have been found to have a marked inhibitory effect on growth of microorganisms. McLaren & Knight (141) found no growth-promoting effect of polylysine for *L. mesenteroides* P-60 in lysine-deficient medium. On the contrary, polylysine proved to be highly toxic to the test organism. Katchalski, Bichowski-Solmnitzski & Volcani (142) observed that many poly α-amino acids were inhibitory to *E. coli* and *M. pyogenes* var. *aureus*. The basic polyamino acids, polylysine, polyornithine, and polyarginine, completely inhibited growth of the test organisms in a synthetic medium, whereas the neutral and acidic polyamino acids, polyalanine, polyaspartic acid, and polyglutamic acid, were without effect. Moreover, the inhibitory effect of polylysine could be readily reversed by polyaspartic and polyglutamic acids.

The inhibitory activity of lysine polypeptides was directly related to molecular weight, the relative toxicities being

$$\text{dilysine} < \text{trilysine} < \text{tetralysine} < \text{polylysine}.$$

The foregoing experiments corroborate the earlier observations of Burger & Stahmann (143) who interpreted their results as indicating a chemical combination between basic polypeptide and the acidic bacterial cell. Similar chemical binding between basic (not acidic) dyes and oxidative enzymes of *E. coli* with a concomitant loss of enzyme activity has also been observed by Quastel & Wheatley (144).

PURINES AND PYRIMIDINES

Lieberman & Kornberg (145, 146) have isolated a soil anaerobe capable of degrading orotic acid to 5-acetic acid hydantoin via the following pathway:

$$\text{orotic acid} \rightleftarrows \text{dihydroorotic acid} \rightleftarrows \text{ureidosuccinic} \rightarrow \text{5-acetic hydantoin.}$$

In contrast to the earlier observed inactivity of synthetic "dihydroorotic acid," the enzymatically synthesized acid was equally as active as orotic acid in supporting the growth of *L. bulgaricus* 09 (147). It appeared from the above data that the synthetic product was not dihydroorotic acid. This proved to be the case when dihydroorotic acid synthesized by a new route was found to possess full microbiological activity for *L. bulgaricus* [Miller, Gordon & Engelhardt (148)]. D-Dihydroorotic acid was shown to be toxic to *L. bulgaricus*, and the toxicity was reversed by the L-isomer. Dihydroorotic acid is also capable of supporting the growth of several other pyrimidine-requiring lactobacilli (149).

Cell suspensions and cell-free extracts of *L. arabinosus* were reported to be capable of converting 5-amino-4-imidazolecarboxamide or its riboside to purines [Weaver & Shive (150)]. The PGA antagonist, aminopterin, was found to inhibit markedly purine synthesis as measured by the disappearance of carboxamide. Thymidine but not thymine or hypoxanthine desoxyriboside reversed the inhibition.

Studies dealing with the purine nutrition of *L. casei* indicate that two distinct pathways may exist for the metabolism of adenine [Elion, Singer & Hitchings (151)]. Evidence supporting this contention is based primarily on apparent differences noted among wild type and 2,6-diaminopurine-and 6-mercaptopurine-resistant strains of *L. casei* with respect to adenine utilization and its inhibition by purine antagonists (151, 152). The diaminopurine-resistant culture differs from the mercaptopurine-resistant one in its sensitivity to mercaptopurine and its insensitivity to adenine antagonists (8-azaadenine and diaminopurine). These observations, in addition to the relative growth-promoting activities of a series of purines for these microorganisms, seem to indicate distinct differences in the purine metabolism of *L. casei*.

The b isomers of adenylic and guanylic acids were demonstrated to be as effective as the free bases in supporting growth of *L. casei* (153). On the other hand, the a isomers were poorly utilized as evidenced by growth and isotope incorporation experiments. The facts that adenine and not its ribotide is able to reverse diaminopurine toxicity, and that adenylic acid b is more active than adenine for the growth of diaminopurine-resistant strain of *L. casei* are offered as evidence in support of the utilization of nucleotides per se (153, 154).

Several experiments during the past year have extended our knowledge of the interconversion of purines in microorganisms. Previous studies with *Torulopsis utilis* indicated that adenine could be utilized for the biosynthesis

of RNA-adenine and guanine, whereas guanine could be incorporated only into RNA-guanine [Kerr, Seraidarian & Brown (155)]. More recent studies by Kerr & Chernigoy (156) in which the effect of purine bases on the incorporation of radioactive formate into RNA[2] purines was observed corroborated the earlier findings. Furthermore, they demonstrated by similar techniques that 2,6-diaminopurine is utilized by *T. utilis* for the synthesis of guanine but not adenine RNA and desoxyribose nucleic acid.

Unlike yeast, the protozoan *Ochromonas malhamensis* was found by Hamilton to utilize adenine, guanine, and to a lesser extent 2,6-diaminopurine for the synthesis of RNA-adenine and guanine (157). On the other hand, the protozoan *T. geleii* can utilize guanine for synthesis of both guanine and adenine nucleotides but can utilize adenine only for synthesis of nucleic acid adenine [Flavin & Graff (158, 159)]. *Trichomonas vaginalis* is apparently unable to convert guanine to adenine (160). These reports indicate the wide differences in purine metabolism among protozoa in particular and among microorganisms in general.

Unknown Growth Factors

A comparatively large number of papers have appeared during the last year indicating the essentiality for growth of a number of crude organic materials or concentrates. Some of these requirements were apparent in chemically defined media but in many cases the well-known growth factors were not tested for activity. Only those experiments in which the readily-available known growth factors were ruled out are discussed in this section.

Veltre, Shorb & Pelczar (161) demonstrated that *L. bifidus* isolated from chicken caecum required an unknown growth factors (s) present in yeast extract, fish solubles, and beef extract. The crystalline disaccharide (galactose and acetylglucosamine) effective in supporting growth of human bifids was inactive for the chick isolates (162).

Yeast extract supplied a factor essential for the growth of *Fusobacterium nucleatum* in a vitamin-free casein hydrolyzate medium. The factor is stable to heat at neutral pH, but is inactivated with 5N acid or alkali at 120°C. Yeast autolyzate increased twofold the early growth of *Brucella melitensis* (164).

Jones & Clifton (165) observed that a chemically-defined medium optimal for *Clostridium perfringens* would not support growth of *C. feseri* unless it was supplemented with enzymatic digest of casein. This requirement may be related to strepogenin and required for reasons discussed earlier with reference to peptide requirement of lactic acid bacteria.

Burton & Lochhead (2, 166) demonstrated that a soil isolate, designated *A. terregens*, required for growth an unknown factor present in liver extract and produced by another *Arthrobacter* (167). Recent studies indicate that the *Arthrobacter* factor is related to ferrichrome and the coprogens (20).

According to Tria & Barnabei (168) growth of *S. cerevisiae* is doubled by the addition of acetone extracts of liver to a synthetic medium. Fractions

active for the yeast were also active in promoting growth of hyperthyroid rats.

Lipid-like substances appear to be required by some protozoa. *Peranema trichophorum* grows in chemically defined media only when they are supplemented with cream or liver digests (41). *Trichomonas foetus* (169) and the Reiter treponeme (170) require serum for growth. In both instances cholesterol is inactive. However, for the treponeme, oleic acid and other unsaturated fatty acids replace the unknown factor.

Van Wagtendonk & Conner (171) demonstrated that the factor in lemon juice required by *Paramecium aurelia* in trace quantities (10 mμg. per ml.) is probably β-sitosterol and can be replaced by several related steroids (171, 172).

The hemoflagellate, *Hereptomonas culicidinum*, was cultivated in a chemically defined medium supplemented with small quantities of liver extract (173). Relatively high levels of PGA could replace liver; citrovorum factor was inactive.

The biosynthesis of riboflavin by *Eremothecium ashbyii* was enhanced greatly in chemically defined media by the addition of yeast extract and vitamin-free casein [Hickey (174)]. Yeast extract could be replaced by oleic acid. However, extraction data indicate that the factor in yeast apparently is not fatty acid.

Camien & Dunn (175) found that the unknown factor required by a mutant of *L. casei* which was present in yeast extract is D-lactic acid. D-phenyllactic acid is also active. The exact function of D-lactic acid in the metabolism of *L. casei* is still unknown.

Several acetic acid bacteria were unable to utilize ethanol, pyruvate, or lactate as sole carbon sources in a medium which supported growth when either acetate or glucose was the sole carbon source (176). Ethanol was utilized only when the medium was supplemented with yeast. Recently, Raghavendra Rao & Stokes (177) have shown the unknown factor to be reducing sugar. Apparently some species of *Acetobacter* are unable to utilize ethanol unless small quantities of reducing sugar are added to "spark" the reaction.

In addition to biotin, an unknown factor in liver or yeast extract is essential for aspartic deaminase activity [Lichstein (178)]. Christman & Williams were able to obtain the cofactor by sulfuric acid degradation of glucose and other carbohydrates (179). More recent experiments by Christman & Williams (180) indicate that none of the known degradation products of glucose is effective in activating the deaminases and that the cofactor does not affect biotin synthesis by cell suspensions of *Bacterium cadaveris*. These authors suggest that the "synthetic" cofactor and the factor in yeast extract and liver are identical.

Antibiotics

Although the mode of action of antibiotics is discussed in detail elsewhere in this volume, consideration will be given here to the nutritional

aspects of antibiotic action. Considerable literature has appeared during the past two years demonstrating the reversal of antibiotic activity by known metabolities. In 1950 Woolley (181) demonstrated that chloramphenicol toxicity is reversed in a noncompetitive manner by phenylalanine. Later it was shown that the antibiotic activity of actathiazic acid is reversed readily by biotin and pimelic acid [Hanes & Sylvester (182)]. Studies on the effect of erythromycin on growth of *Corynebacterium diphtheriae* indicated that such toxicity is reversed by β-alanine, carnosine, and pantothenic acid [Brown & Emerson (183)].

Recently chlortetracycline (aureomycin) toxicity was found to be reversed readily by riboflavin [Foster & Pittillo (184)]. Chlorotetracycline apparently inhibits utilization of riboflavin by riboflavinless strains of *B. subtilis* and *Aspergillus niger* and biosynthesis of the vitamin by *A. aerogenes* and *B. subtilis*. Terramycin functions in a manner analogous to that of chlortetracycline (184).

Although cross-resistance occurs among microorganisms between streptomycin and dihydrostreptomycin, Pittillo & Foster (185) were able to demonstrate that the modes of action of these antibiotics are distinctly different. Whereas streptomycin toxicity for *A. aerogenes* was reversed by guanosine, guanylic acid, cytosine, or thymine, these compounds had no appreciable effect on dihydrostreptomycin toxicity. Against this latter antibiotic, phenylalanine was highly effective, but was inactive in reversing streptomycin toxicity.

The antibacterial activity of ascosin, a polyene-type antifungal agent (conjugated unsaturated bonds in a chain structure), for *S. cerevisiae* was antagonized markedly by the unsaturated long chain fatty acids, oleic, linoleic, and linolenic acids [Hickey (186)]. However, the saturated fatty acids through stearic acid were ineffective. Two other polyene-type antibiotics, prodigiosin and fungicidin, also were antagonized by oleic acid.

Since Miller & Bohnhoff (187) first reported the occurrence of streptomycin-dependent meningococci several other streptomycin-dependent microorganisms have been obtained. Sevag & Rosanoff (188) have demonstrated that streptomycin-dependent strains of *E. coli* can be grown in the absence of streptomycin provided the medium is supplemented with bacterial extracts or a fraction from bovine blood serum.

The above studies provide strong evidence in favor of the metabolite-antimetabolite hypothesis of antibiotic action.

LITERATURE CITED

1. Stokes, J. L., *Ann. Rev. Microbiol.*, **6**, 29–48 (1952)
2. Cheldelin, V. H., and King, T. E., *Ann. Rev. Microbiol.*, **7**, 113–42 (1953)
3. Borek, E., and Waelsch, H., *J. Biol. Chem.*, **190**, 191–96 (1951)
4. Hills, G. M., and Spurr, E. D., *J. Gen. Microbiol.*, **6**, 64–73 (1952)
5. Campbell, L. L., and Williams, O. B., *J. Bact.*, **65**, 141–45 (1953)
6. Kocher, V., *Intern. Z. Vitaminforsch.*, **20**, 369–74 (1949)
7. Koditschek, L. K., Hendlin, D., and Woodruff, H. B., *J. Biol. Chem.*, **179**, 1093–1102 (1949)

8. Hendlin, D., and Koditschek, L. K. (Unpublished data)
9. Richardson, G. M., *Biochem. J. (London)*, **30,** 2184–90 (1936)
10. Stone, D., and Hoberman, H. D., *J. Biol. Chem.*, **202,** 203–12 (1953)
11. Brown, J. W., and Binkley, S. B., *Federation Proc.*, **12,** 183 (1953)
12. Lyman, C. M., Moseley, O., Wood, S., Butler, B., and Hale, F., *J. Biol. Chem.*, **167,** 177–87 (1947)
13. Broquist, H. P., and Snell, E. E., *J. Biol. Chem.*, **188,** 431–44 (1951)
14. Caswell, M. C., and Hendlin, D. (Unpublished data)
15. Hesseltine, C. W., Pidacks, C., Whitehill, A. R., Bohonos, N., Hutchings, B. L., and Williams, J. H., *J. Am. Chem. Soc.*, **74,** 1362 (1952)
16. Hesseltine, C. W., Whitehill, A. R., Pidacks, C., Tenhagen, M., Bohonos, N., Hutchings, B. L., and Williams, J. H., *Mycologia*, **45,** 7–19 (1953)
17. Pidacks, C., Whitehill, A. R., Pruess, L. M., Hesseltine, C. W., Hutchings, B. L., Bohonos, N., and Williams, J. H., *J. Am. Chem. Soc.*, **75,** 6064–65 (1953)
18. Neilands, J. B., *J. Am. Chem. Soc.*, **74,** 4864–47 (1952)
19. Neilands, J. B., *Federation Proc.*, **12,** 250 (1953)
20. Neilands, J. B., *J. Biol. Chem.*, **205,** 643–46 (1953)
21. Neilands, J. B., *J. Biol. Chem.*, **205,** 647–50 (1953)
22. Webb, M., *J. Gen. Microbiol.*, **2,** 275–87 (1948)
23. Webb, M., *J. Gen. Microbiol.*, **3,** 410–24 (1949)
24. Rochford, E. J., and Mandle, R. J., *J. Bact.*, **66,** 554–60 (1953)
25. Sheffner, A. L., and Grabow, J., *J. Bact.*, **65,** 192–95 (1953)
26. Camien, M. N., and Dunn, M. S., *Proc. Soc. Exptl. Biol. Med.*, **81,** 564–66 (1952)
27. Eades, C. H., Jr., and Womack, M., *J. Bact.*, **65,** 322–25 (1953)
28. Walker, J. B., *Arch. Biochem. and Biophys.*, **46,** 1–11 (1953)
29. Lewis, S. Z., Lucchesi, P. J., and Vance, J. E., *J. Am. Chem. Soc.*, **75,** 6058 (1953)
30. Nicholas, D. J. D., Nason, A., and McElroy, D., *Nature*, **172,** 34 (1953)
30a. DeRenzo, E. C., Kaleita, E., Heytler, P., Oleson, J. J., Hutchings, B. L., and Williams, J. H., *J. Am. Chem. Soc.*, **75,** 753 (1953)
31. MacLeod, R. A., and Snell, E. E., *Ann. N. Y. Acad. Sci.*, **52,** 1249–59 (1950)
32. MacLeod, R. A., *J. Bact.*, **67,** 23–26 (1954)
33. Hutner, S. H., Provasoli, L., Stokstad, E. L. R., Hoffmann, C. E., Bett, M., Franklin, A. L., and Jukes, T. H., *Proc. Soc. Exptl. Biol. Med.*, **70,** 118–20 (1949)
34. Provasoli, L., and Pintner, I. J., *Ann. N. Y. Acad. Sci.*, **56,** 839–51 (1953)
35. Hendlin, D., and Woodruff, H. B., *Abstracts Am. Chem. Soc., 116th Meeting*, 33A (Atlantic City, N. J., September, 1949)
36. Robbins, W. J., Hervey, A., and Stebbins, M. E., *Nature*, **170,** 845–46 (1952)
37. Robbins, W. J., Hervey, A., and Stebbins, M. E., *Ann. N. Y. Acad. Sci.*, **56,** 818–30 (1953)
38. Mollin, D. L., and Ross, G. I. M., *J. Clin. Pathol.*, **6,** 54–61 (1953)
39. Hutner, S. H., Provasoli, L., and Filfus, J., *Ann. N. Y. Acad. Sci.*, **56,** 852–63 (1953)
40. Ford, J. E., *Brit. J. Nutrition*, **7,** 299–306 (1953)
41. Storm, J., and Hutner, S. H., *Ann. N. Y. Acad. Sci.*, **56,** 901–9 (1953)
42. Barber, F. W., Baile, D. L., Troescher, C. B., and Huhtanen, C. N., *Ann. N. Y. Acad. Sci.*, **56,** 863–69 (1953)
43. Hendlin, D., and Soars, M. H., *J. Bact.*, **62,** 633–38 (1951)

44. Hendlin, D., *Ann. N. Y. Acad. Sci.*, **56**, 870–71 (1953)
45. Oginsky, E. L., and Smith, P. H., *J. Bact.*, **65**, 183–86 (1953)
46. Corbett, J. J., *J. Bact.*, **64**, 889–90 (1952)
47. Bardos, T. J., and Gordon, H. L., *J. Am. Chem. Soc.*, **75**, 2019–20 (1953)
48. Davis, R. L., and Chow, B. F., *Science*, **115**, 351–52 (1952)
49. Gordon, H. L., Heenan, E. F., and Bardos, T. J., *Abstracts Am. Chem. Soc., 124th Meeting*, 7C (Chicago, Ill., September, 1953)
50. Daniel, L. J., and Gray, L. F., *Proc. Soc. Exptl. Biol. Med.*, **83**, 487–90 (1953)
51. Beiler, J. M., Moss, J. N., and Martin, G. J., *Science*, **114**, 122–23 (1951)
52. Villela, G. G., and Abreu, L. A., *Science*, **115**, 205 (1952)
53. Hendlin, D., and Wall, J. C., *J. Bact.*, **67**, 38–40 (1954)
54. Rodwell, A. W., *J. Gen. Microbiol.*, **8**, 238–47 (1953)
55. Dewey, V. C., and Kidder, G. W., *J. Gen. Microbiol.*, **9**, 445–53 (1953)
56. Nichol, C. A., Zakrzewski, S. F., and Welch, A. D., *Proc. Soc. Exptl. Biol. Med.*, **83**, 272–77 (1953)
57. Hutchison, D. J., and Burchenal, J. H., *Proc. Am. Assoc. Cancer Research*, **1**, 26 (1953)
58. Dewey, V. C., and Kidder, G. W., *Proc. Soc. Exptl. Biol. Med.*, **83**, 586–88 (1953)
59. Heinrich, M. R., Dewey, V. C., and Kidder, G. W., *J. Am. Chem. Soc.*, **75**, 5425–26 (1953)
60. Foley, G. E., *Proc. Soc. Exptl. Biol. Med.*, **83**, 733–39 (1953)
61. Foley, G. E., *Proc. Soc. Exptl. Biol. Med.*, **83**, 740–42 (1953)
62. Foley, G. E., and Watson, P. L., *Proc. Soc. Exptl. Biol. Med.*, **83**, 742–48 (1953)
63. Burchenal, J. H., Waring, G. B., and Hutchison, D. J., *Proc. Soc. Exptl. Biol. Med.*, **82**, 536–38 (1953)
64. Broquist, H. P., Kohler, A. R., Hutchison, D. J., and Burchenal, J. H., *J. Biol. Chem.*, **202**, 59–66 (1953)
65. Brown, D. J., *J. Chem. Soc.*, **1644**–45 (1953)
66. Nimmo-Smith, R. H., and Brown, D. J., *J. Gen. Microbiol.*, **9**, 536–44 (1953)
67. Sauberlich, H. E., and Baumann, C. A., *J. Biol. Chem.*, **176**, 165–73 (1948)
68. Felton, E. A., and Niven, C. F., *J. Bact.*, **65**, 482–83 (1953)
69. Bardos, T. J., Bond, T. J., Humphreys, J., and Shive, W., *J. Am. Chem. Soc.*, **71**, 3852 (1949)
70. Hendlin, D., Koditschek, L. K., and Soars, M. H., *J. Bact.*, **65**, 466–71 (1953)
71. Bond, T. J., *Science*, **117**, 563–64 (1953)
72. Flynn, E. H., Bond, T. J., Bardos, T. J., and Shive, W., *J. Am. Chem. Soc.*, **73**, 1979–82 (1951)
73. Nichol, C. A., and Welch, A. D., *Proc. Soc. Exptl. Biol. Med.*, **74**, 52–45 (1950)
74. Dietrich, L. S., Monson, W. J., and Elvehjem, C. A., *Proc. Soc. Exptl. Biol. Med.*, **77**, 93–96 (1951)
75. Nichol, C. A., and Welch, A. D., *Proc. Soc. Exptl. Biol. Med.*, **74**, 403–11 (1950)
76. Wold, F., and Sirny, R. J., *Federation Proc.*, **12**, 292 (1953)
77. Lansford, E. M., and Shive, W., *Arch. Biochem. and Biophys.*, **38**, 353–55 (1952)
78. Maas, W. K., and Vogel, H. J., *J. Bact.*, **65**, 388–93 (1953)
79. King, T. E., Stearman, R. L., and Cheldelin, V. H., *J. Am. Chem. Soc.*, **70**, 3969 (1948)
80. Pomper, S., *J. Bact.*, **64**, 353–61 (1952)
81. Purko, M., Nelson, W. O., and Wood, W. A., *J. Dairy Sci.*, **34**, 874–78 (1951)

82. Purko, M., Nelson, W. O., and Wood, W. A., *J. Bact.*, **66,** 561–67 (1953)
83. Ryan, F. J., Kunin, C., and Ballentine, R., *J. Bact.*, **65,** 434–39 (1953)
84. Pittillo, R. F., and Foster, J. W., *J. Bact.*, **67,** 53–57 (1954)
85. Brown, G. M., and Snell, E. E., *J. Am. Chem. Soc.*, **75,** 2782–83 (1953)
86. King, T. E., and Cheldelin, V. H., *Proc. Soc. Exptl. Biol. Med.*, **84,** 591–93 (1953)
87. Heyl, D., Luz, E., Harris, S. A., and Folkers, K., *J. Am. Chem. Soc.*, **74,** 414–19 (1952)
88. Rabinowitz, J. C., and Snell, E. E., *J. Am. Chem. Soc.*, **75,** 998–99 (1953)
89. Martin, G. J., Avakian, S., and Moss, J., *J. Biol. Chem.*, **174,** 495–500 (1948)
90. Rabinowitz, J. C., and Snell, E. E., *Arch. Biochem. and Biophys.*, **43,** 399–407 (1953)
91. Rabinowitz, J. C., and Snell, E. E., *Arch. Biochem. and Biophys.*, **43,** 408–15 (1953)
92. Yoneda, M., Kato, N., and Okajima, M., *Nature*, **170,** 803 (1952)
93. Yoneda, M., and Asano, N., *Science*, **117,** 277–79 (1953)
94. Boone, I. U., and Woodward, K. T., *Proc. Soc. Exptl. Biol. Med.*, **84,** 292–96 (1953)
95. Moulder, J. W., and Woods, D. D., *J. Gen. Microbiol.*, **9,** *iv–v* (1953)
96. Campbell, L. L., and Williams, O. B., *J. Bact.*, **65,** 146–47 (1953)
97. Williams, R. J., Eakin, R. E., Beerstecher, E., and Shive, W., *The Biochemistry of B Vitamins*, 88 (Reinhold Publishing Co., New York, N. Y., 744 pp., 1950)
98. Koser, S. A., Wright, M. H., and Dorfman, A., *Proc. Soc. Exptl. Biol. Med.*, **51,** 204–5 (1942)
99. Broquist, H. P., and Snell, E. E., *Arch. Biochem. and Biophys.*, **46,** 432–42 (1953)
100. Lampen, J. O., Jones, M. J., and Perkins, A. B., *Arch. Biochem.*, **13,** 33–45 (1947)
101. Sourkes, T. L., and Trano, Y., *Arch. Biochem. and Biophys.*, **42,** 321–26 (1953)
102. Pomper, S., *J. Bact.*, **65,** 666–70 (1953)
103. Waelsch, H., Owades, P., Miller, H. K., and Borek, E., *J. Biol. Chem.*, **166,** 273–81 (1946)
104. Elliot, W. H., and Gale, E. F., *Nature*, **161,** 129–30 (1948)
105. Krask, B. J., *J. Bact.*, **66,** 374 (1953)
106. Kalan, E., and Ceithaml, J., *Federation Proc.*, **12,** 228 (1953)
107. Davis, B. D., and Mingioli, E. S., *J. Bact.*, **60,** 17–28 (1950)
108. Shapiro, S. K., *J. Bact.*, **65,** 310–12 (1953)
109. Horowitz, N. H., *Advances in Genetics*, **3,** 33–71 (1950)
110. Simmonds, S., *J. Biol. Chem.*, **174,** 717–22 (1948)
111. Teas, H. J., *J. Bact.*, **59,** 93–104 (1950)
112. Schwartz, M., and Shapiro, S. K., *J. Bact.*, **67,** 98–102 (1954)
113. Cohn, M., Cohen, G., and Monod, J., *Compt. rend.*, **236,** 746–48 (1953)
114. Wijesundera, S., and Woods, D. D., *Biochem. J.* (*London*), **55,** viii (1953)
115. Doudney, C. O., and Wagner, R. P., *Proc. Natl. Acad. Sci. U. S.*, **39,** 1043–52 (1953)
116. Vogel, H. J., *Proc. Natl. Acad. Sci. U. S.*, **39,** 578–83 (1953)
117. Rudman, D., and Meister, A., *J. Biol. Chem.*, **200,** 591–604 (1953)
118. Umbarger, H. E., and Mueller, J. H., *J. Biol. Chem.*, **189,** 277–85 (1951)
119. Bonner, D., *J. Biol. Chem.*, **166,** 545–54 (1946)

120. Umbarger, H. E., *J. Bact.*, **66**, 328–32 (1953)
121. Rowley, D., *J. Gen. Microbiol.*, **9**, 37–43 (1953)
122. Ayengar, P., and Roberts, E., *Proc. Soc. Exptl. Biol. Med.*, **79**, 476–81 (1952)
123. Roberts, E., and Hunter, P. F., *Proc. Soc. Exptl. Biol. Med.*, **83**, 720–21 (1953)
124. Shive, W., and Macow, J. J., *J. Biol. Chem.*, **162**, 451–62 (1946)
125. Buston, H. W., Churchman, J., and Bishop, J., *J. Biol. Chem.*, **204**, 665–68 (1953)
126. Davis, B. D., *Experientia*, **6**, 41–50 (1950)
127. Salamon, I. I., and Davis, B. D., *J. Am. Chem. Soc.*, **75**, 5567–71 (1953)
128. Weiss, U., Davis, B. D., and Mingioli, E. S., *J. Am. Chem. Soc.*, **75**, 5572–76 (1953)
129. Shiguera, H., Sprinson, D. B., and Davis, B. D., *Federation Proc.*, **12**, 458 (1953)
130. Thomas, R. C., Cheldelin, V. H., Christensen, B. E., and Wang, C. H., *J. Am. Chem. Soc.*, **75**, 5554–56 (1953)
131. Cutinelli, C., Ehrensvard, G., Reio, L., Saluste, E., and Stjernholm, R., *Acta Chem. Scand.*, **5**, 353–71 (1951)
132. Beerstecher, E., and Shive, W., *J. Biol. Chem.*, **167**, 49–52 (1947)
133. Simmonds, S., Tatum, E. L., and Fruton, J. S., *J. Biol. Chem.*, **169**, 91–101 (1947)
134. Bergmann, E. D., Sicher, S., and Volcani, B. E., *Biochem. J.* (*London*), **54**, 1–13 (1953)
135. Kihara, H., and Snell, E. E., *J. Biol. Chem.*, **197**, 791–800 (1952)
136. Kihara, H., Klatt, O. A., and Snell, E. E., *J. Biol. Chem.*, **197**, 801–7 (1952)
137. Prescott, J. M., Peters, V. J., and Snell, E. E., *J. Biol. Chem.*, **202**, 533–40 (1953)
138. Simmonds, S., and Fruton, J. S., *J. Biol. Chem.*, **174**, 705–15 (1948)
139. Hirsch, M. L., and Cohen, G. N., *Biochem. J.* (*London*), **53**, 25–30 (1953)
140. Peters, V. J., Prescott, J. M., and Snell, E. E., *J. Biol. Chem.*, **202**, 521–32 (1953)
141. McLaren, A. D., and Knight, C. A., *J. Biol. Chem.*, **204**, 417–22 (1953)
142. Katchalski, E., Bichowski-Slomnitzki, L., and Volcani, B. E., *Biochem. J.* (*London*), **55**, 671–80 (1953)
143. Burger, W. C., and Stahmann, M. A., *Arch. Biochem. and Biophys.*, **39**, 27–36 (1952)
144. Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.* (*London*), **25**, 629–38 (1931)
145. Lieberman, I., and Kornberg, A., *Federation Proc.*, **12**, 239 (1953)
146. Lieberman, I., and Kornberg, A., *Biochim. et Biophys. Acta*, **12**, 223–34 (1953)
147. Spicer, D. S., Liebert, K. V., Wright, L. D., and Huff, J. W., *Proc. Soc. Exptl. Biol. Med.*, **79**, 587–88 (1952)
148. Miller, C. S., Gordon, J. T., and Engelhardt, E. L., *J. Am. Chem. Soc.*, **75**, 6086–87 (1953)
149. Wright, L. D., Driscoll, C. A., Miller, C. S., and Skeggs, H. R., *Proc. Soc. Exptl. Biol. Med.*, **84**, 716–19 (1953)
150. Weaver, J. M., and Shive, W., *J. Am. Chem. Soc.*, **75**, 4628 (1953)
151. Elion, G. B., Singer, S., and Hitchings, G. H., *J. Biol. Chem.*, **204**, 35–41 (1953)
152. Elion, G. B., Singer, S., Hitchings, G. H., Balis, M. E., and Brown, G. B., *J. Biol. Chem.*, **202**, 647–54 (1953)
153. Balis, M. E., Levin, D. H., Brown, G. B., Elion, G. B., Vanderwerff, H., and Hitchings, G. H., *J. Biol. Chem.*, **200**, 1–6 (1953)

154. Elion, G. B., Vanderwerff, H., Hitchings, G. H., Balis, M. E., Levin, D. H., and Brown, G. B., *J. Biol. Chem.*, **200,** 7–16 (1953)
155. Kerr, S. E., Seraidarian, K., and Brown, G. B., *J. Biol. Chem.*, **188,** 207–16 (1951)
156. Kerr, S. E., and Chernigoy, F., *J. Biol. Chem.*, **200,** 887–94 (1953)
157. Hamilton, L., *Ann. N. Y. Acad. Sci.*, **56,** 961–68 (1953)
158. Flavin, M., and Graff, S., *J. Biol. Chem.*, **191,** 55–61 (1951)
159. Flavin, M., and Graff, S., *J. Biol. Chem.*, **192,** 485–88 (1951)
160. Sprince, H., Goldberg, R., Kucker, G., and Lowy, R. S., *Ann. N. Y. Acad. Sci.*, **56,** 1016–27 (1953)
161. Veltre, F. A., Shorb, M. A., and Pelczar, M. J., Jr., *Proc. Soc. Exptl. Biol. Med.*, **83,** 284–86 (1953)
162. Tomarelli, R. M., Linden, E., and Bernhart, F. W., *Federation Proc.*, **12,** 431 (1953)
163. Omata, R. R., *J. Bact.*, **65,** 326–29 (1953)
164. Sanders, T. H., Higuchi, K., and Brewer, C. R., *J. Bact.*, **66,** 294–99 (1953)
165. Jones, L. W., and Clifton, C. E., *J. Bact.*, **65,** 560–64 (1953)
166. Burton, M. O., and Lochhead, A. G., *Can. J. Botany*, **31,** 145–52 (1953)
167. Lochhead, A. G., and Burton, M. O., *Can. J. Botany*, **31,** 7–23 (1953)
168. Tria, E., and Barnabei, O., *Nature*, **172,** 203–4 (1953)
169. Clausen, J. K., *Nature*, **171,** 1067 (1953)
170. Oyama, V. I., Steinman, H. G., and Eagle, H., *J. Bact.*, **65,** 609–16 (1953)
171. Van Wagtendonk, W. J., and Conner, R. L., *Federation Proc.*, **12,** 283 (1953)
172. Van Wagtendonk, W. J., Conner, R. L., Miller, C. A., and Rao, M. R. R., *Ann. N. Y. Acad. Sci.*, **56,** 929–37 (1953)
173. Cowperthwaite, J., Weber, M. M., Packer, L., and Hutner, S. H., *Ann. N. Y. Acad. Sci.*, **56,** 972–81 (1953)
174. Hickey, R. J., *J. Bact.*, **66,** 27–33 (1953)
175. Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, **201,** 621–28 (1953)
176. Raghavendra Rao, M. R., and Stokes, J. L., *J. Bact.*, **65,** 405–12 (1953)
177. Raghavendra Rao, M. R., and Stokes, J. L., *J. Bact.*, **66,** 634–38 (1953)
178. Lichstein, H. C., *Vitamins and Hormones*, **9,** 27–74 (1951)
179. Christman, J. F., and Williams, V. R., *J. Bact.*, **63,** 107–10 (1952)
180. Williams, V. R., and Christman, J. F., *J. Bact.*, **65,** 238–44 (1953)
181. Woolley, D. D., *J. Biol. Chem.*, **185,** 293–305 (1950)
182. Hanes, M. E., and Sylvester, J. C., *Antibiotics & Chemotherapy*, **2,** 399–408 (1952)
183. Brown, R. G., and Emerson, G. A., *Federation Proc.*, **12,** 304–5 (1953)
184. Foster, J. W., and Pittillo, R. F., *J. Bact.*, **66,** 478–86 (1953)
185. Pittillo, R. F., and Foster, J. W., *Proc. Soc. Exptl. Biol. Med.*, **84,** 568–70 (1953)
186. Hickey, R. J., *Arch. Biochem. and Biophys.*, **46,** 331–36 (1953)
187. Miller, P. C., and Bohnhoff, M., *Science*, **105,** 620–21 (1947)
188. Sevag, M. G., and Rosanoff, E. I., *Arch. Biochem. and Biophys.*, **45,** 233–34 (1953)

METABOLISM OF MICROORGANISMS[1,2]

By Jack J. R. Campbell

Department of Dairying, The University of British Columbia, Vancouver, B. C., Canada

An attempt has been made to report on all aspects of microbial metabolism in this review. The pitfalls awaiting anyone claiming to be qualified to undertake such an endeavor may be apparent. However, the increasing number of publications devoted to comprehensive reviews or symposia on some aspect of the metabolism of microorganisms would seem to point to the need of an annual review which is not comprehensive but which attempts to bring together the vast variety of contributions made during the year. Because of the diversity of topics it was not feasible to grace each subject with a subheading. As a result the oxidation of glycerol and cyclitols is discussed with the oxidation of carbohydrates while the oxidation of phenol is included in the discussion of aromatic acids.

Adaptive Enzyme Formation

After passing through a period in which no generally acceptable theory of enzyme synthesis was evident it would appear that, largely as a result of the brilliant work of the French group, a workable, generally applicable hypothesis of the mechanism of adaptive enzyme formation has taken shape.

Cohn & Capdupuy (1) have shown that the earlier explanation of the diauxie effect, which was contradicted by the concept of simultaneous adaptatation, was in error and that in reality glucose inhibits adaptive β-galactozymase formation by interfering with the passage of the inductor across the bacterial cell membrane. Making use of the fact that *Escherichia coli* bacteriophage will multiply in and lyse only those cells which are able to metabolize and gain energy from the added substrate, Benzer (2) was able to show that adaptive production of β-galactozymase occurred to approximately the same extent in all or almost all cells in the culture. Therefore the sigmoid curve obtained during induction is not attributable to a selection

[1] The survey of the literature pertaining to this review was concluded in December, 1953.

[2] The following abbreviations are used: ADP for adenosinediphosphate; AMP for adenosinemonophosphate; ATP for adenosinetriphosphate; CoA for coenzyme A; acetyl-P for acetyl phosphate; DPN for diphosphopyridine nucleotide; DPNH or DPNH[2] for diphosphopyridine nucleotide (reduced form); DPT for thiamine pyrophosphate; P for inorganic phosphate; FAD for flavin-adenine-dinucleotide; FMN for flavin-adenine-mononucleotide; NMN for nicotinamide mononucleoitide; TCA for tricarboxylic acid; TPN for triphosphopyridine nucleotide; TPNH for triphosphopyridine nucleotide (reduced form); UDP for uridine diphosphate; UDPG for uridine diphosphate glucose.

phenomenon. Furthermore it has been demonstrated that the sigmoid curve is the result of the presence of a number of limiting factors. These factors were shown by Porter *et al.* (3, 4) to be the concentration of the inductor, the nature of the nitrogen source, the duration of the assimilation phase, oxygen tension, and temperature. When the limitations imposed by these factors were removed the time course of induced β-galactosidase formation was strictly linear. Siminovitch & Jacob (5) found that a lysogenic strain of *E. coli* K12 adaptively formed β-galactozymase at normal rate coincident with bacteriophage formation. This observation is in marked contrast with the reports [Monod & Wollman (6)] of the complete inhibition by phage formation in phage sensitive strains of the same organism but is in agreement with Jacob's (7) earlier observations on the ability of lysogenic strains of *Pseudomonas aeruginosa* to form adaptive enzymes during bacteriophage multiplication.

Cohn & Torriani (8) found that the induction of the β-galactosidase of *E. coli* resulted in the formation of a new protein which could be identified by its specific immunological reactions. This new protein (G_z) was identical with β-galactosidase. Extracts from cells which had not been induced to produce the enzyme did not contain G_z but did contain a protein (P_z) which cross-reacted strongly with the antiserum to G_z. These workers also showed that in a growing culture the synthesis of G_z coincided with a decrease in the P_z content of the cells (9). Attempts to induce nitrogen-starved cells of *E. coli* resulted in no formation of G_z. P_z remained at a normal level over a 24 hr. period of starvation. These data indicate that synthetic reactions involving nitrogen are necessary for the conversion of P_z to G_z and that P_z is the noninduced precursor of G_z. Additional support for the postulated role of P_z was obtained with the finding that species of Enterobacteriaceae which possessed the P_z protein had the ability to form β-galactozymase whereas species which did not possess P_z did not have the ability to synthesize β-galactosidase. It was also shown that a strain of *Lactobacillus bulgaricus* and of *Saccharomyces fragilis* which did possess β-galactozymase did not possess P_z. However, the β-galactozymase of these two organisms was shown to be enzymatically and immunologically unrelated to the *E. coli* β-galactozymase (14).

Halvorson & Spiegelman (10) found that in the presence of an amino acid analogue such as *p*-fluorophenylalanine growth of *Saccharomyces cerevisiae* was almost completely stopped and adaptive formation of the maltose-splitting enzyme was only 27 per cent of normal. This inhibition could be completely and specifically reversed by the addition of the corresponding homologous amino acid. By following the disappearance of eight representative animo acids from the free amino acid pool, it was shown that the presence of *p*-fluorophenylalanine prevented the incorporation of any of these free amino acids into insoluble nitrogenous components. By exposing cells to varying degrees of starvation it was shown that the capacity of the organism to synthesize maltozymase was a function of the level of the free

amino acid pool (11). It can be concluded that the synthesis of an adaptive enzyme requires the participation of a very broad spectrum of amino acids. Confirmation and extension of these data were obtained by Monod, Pappenheimer & Cohen-Bazire (12) who demonstrated that the formation of β-galactosidase by *E. coli* could occur only under conditions allowing the synthesis of new protein. Mutants which were unable to synthesize individual amino acids were not able to form β-galactosidase unless additions of the particular amino acid were made. It was, therefore, concluded that during the synthesis of an enzyme such as β-galactosidase (G_s) of *E. coli* the following reaction takes place:

$$P_s + \text{amino acids} \rightarrow G_s \qquad 1.$$

Continuing their work with β-galactosidase, Monod, Cohen-Bazire & Cohn (13) found that the formation of the enzyme could be induced by a variety of compounds possessing an intact galactosidic radical. However, some compounds such as methyl, α-galactoside and alkyl and aryl β-galactosides had the ability to induce the formation of the enzyme but were not hydrolyzed by β-galactosidase. In addition the inductivity, in general, was independent of the affinity for the enzyme. A few substances such as phenyl-β-thiogalactoside had a strong affinity for the enzyme but had no ability to induce its synthesis. These workers concluded that their observations were incompatible with all the hypotheses which are based on the assumption that induction is connected with the activity of the enzyme or is initiated by the formation of a complex between the enzyme and substrate. It was postulated that all compounds which induce the synthesis of β-galactosidase must be capable of being metabolized to a common intermediate. This intermediate in turn would then combine with some cellular constituent which is not the enzyme itself.

Similar conclusions based on studies of penicillinase production, were arrived at by Pollock who found that penicillinase was formed continuously at constant speed in the presence of a mere trace of penicillin (14). Since the trace of penicillin would have been destroyed almost immediately, it was concluded that the penicillin reacted with some cellular constituent and thus contributed to the formation of a complex or compound which is not attacked by the enzyme but which acts as a catalyst and determines the rate of penicillinase formation. Pollock (15) has calculated that if each molecule of fixed penicillin induced the formation of enzyme by actually combining stoichiometrically with one molecule of enzyme precursor, the penicillinase produced would have to have a turnover number 50 times that of catalase in order to account for the observed rate of penicillin destruction. The improbability of penicillinase being so active an enzyme has led Pollock to conclude that the penicillin complex must act as a catalyst in the induction of enzyme synthesis. On the basis of the information at hand Pollock has proposed a tentative scheme of adaptive enzyme formation (see Fig. 1). In this scheme the organizer would catalyze the formation of a specific enzyme

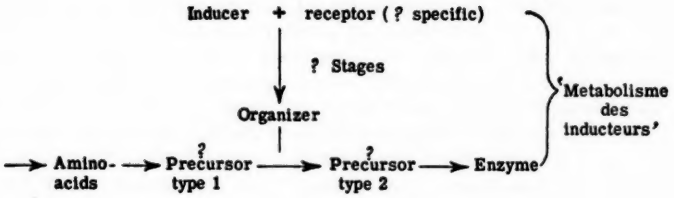


Fig. 1. Pollock's scheme of Adaptive Enzyme Formation.

precursor from a precursor with limited specificity, and the conversion would require the participation of a broad spectrum of free amino acids. An example of precursor type 1 would be the P_s protein of *E. coli*. The organizer is pictured as being a template, a metabolic cycle, or a complex comprising several different substances. The essential characteristics of the organizer as defined by Pollock (15) are that it is distinct from but reflects the specificity of the inducer and that it acts as a catalyst in enzyme formation. It would be a molecule or system of varying stability, and where the continued presence of substrate has been shown to be necessary for enzyme synthesis the organizer is considered to be very unstable. A stable organizer would result in the continued synthesis of enzyme long after the removal of substrate. Cohn & Monod (16) have proposed a very similar though more detailed scheme. Porter *et al.* (4) have also come forward with a scheme that is basically the same.

It is compatible with these theories of enzyme induction which involve the metabolism of the inductor that, depending on conditions, the same compound might act as an inhibitor (analogue) or as an inductor. This phenomenon has now been observed during the synthesis of a number of enzymes, both adaptive and constitutive, and points to the essential similarity in the mechanism of synthesis of these two classes of enzymes (17, 18, 19).

Adaptive formation of benzoic acid oxidizing enzymes of a *Mycobacterium* was induced by a variety of benzoic acid derivatives, some of which were not substrates for the enzyme [Bernheim (20)]. Benzoate oxidation by the adaptive enzyme system of *P. aeruginosa* was necessarily accompanied by NH_3 assimilation, indicating the formation of a very unstable component of the enzyme system [Bernheim & DeTurk (21)].

Carbohydrate

Hydrogen transport.—Kaplan *et al.* (22) continued their studies of the transhydrogenase enzyme of *Pseudomonas fluorescens* and in addition extended their results to include several strains of *Azotobacter*. The enzyme catalyzes the reaction:

$$TPNH + DPN \rightleftarrows TPN + DPNH \qquad 2.$$

It would appear that this reaction is unusual in several respects. Increasing the TPN[2] level greatly inhibited the reversal of the reaction in spite of the fact that TPN was one of the reactants of this reverse reaction. Adenylic acid or its derivatives tended to overcome this inhibition apparently by a competitive antagonism. The conclusion was that there are several sites on the enzyme where TPN may attach itself. One of these sites, which is specific for the *a*-phosphoadenosine derivatives and is considered the point of activation, forms an active complex with all of these derivatives excepting TPN. This explains the dual role of adenylic acid. First as a direct activator of the enzyme and secondly as an analogue of TPN antagonizing the inhibitory effect of TPN on the reversal of the reaction. Too high a concentration of adenylic acid *a*, however, interfered with the reaction between TPN and its other site of attachment and so inhibited the rate of reversibility. The purified transhydrogenase of *P. fluorescens* has been found to catalyze additional reactions involving the following pairs of compounds: DPNH—desamino DPN, TPNH—desamino TPN, TPNH—NMN, and DPNH—NMN. By the use of C^{14}-nicotinamide-labeled DPN[2] Kaplan *et al.* (23) were able to show that the transdehydrogenase reaction definitely involves hydrogen and electron transfer. It was also found that the enzyme catalyzes an interaction between the oxidized and reduced forms of DPN causing an exchange of hydrogen and electrons.

$$DPNH\ (labeled) + DPN \rightleftarrows DPN\ (labeled) + DPNH \qquad 3.$$

It was suggested that the importance of this reaction might be connected with coupled phosphorylation reactions. According to the reasoning of these workers the uptake of phosphate associated with the oxidation of reduced DPN appears to be a property only of the bound pyridine nucleotides of mitochondria. By promoting the transfer reaction the transdehydrogenase would allow soluble DPNH [2] to reduce DPN of the mitochondria and so make possible the coupling of the reaction with phosphate.

Some excellent experimental work by Fisher *et al.* (24) has added greatly to our understanding of the enzymatic transfer of hydrogen by the pyridine nucleotides. Making use of deuterium labeled ethyl alcohol they studied the transfer of hydrogen catalyzed by yeast alcohol dehydrogenase.

$$CH_3CD_2OH + DPN \rightarrow CH_3CDO + DPND + H^+ \qquad 4.$$

When monodeuterium reduced DPN (DPND), prepared enzymatically by this reaction, was reoxidized enzymatically in the presence of acetaldehyde the oxidized DPN contained no excess deuterium. The enzymatic oxidation and reduction of the pyridine nucleotide is therefore stereospecific, and the same hydrogen or deuterium atom which is added to the DPN during reduction is removed during oxidation. The C—H bond was cleaved six times as rapidly as C—D and so the fact that only deuterium was exchanged

in the above reaction proves conclusively that the reaction is truly stereospecific. In contrast when monodeuterium reduced DPN, prepared by the chemical reduction of DPN, was reoxidized by acetaldehyde and alcohol dehydrogenase, the oxidized DPN formed still contained about 0.44 deuterium atoms per molecule. It is evident, therefore, that the chemical reduction was not stereospecific. In addition, when DPN was reduced by ordinary ethyl alcohol plus alcohol dehydrogenase in a medium of pure D_2O the reduced DPN contained no excess deuterium. The enzymatic transfer of hydrogen or deuterium is, therefore, directly from the alcohol to the coenzyme.

Saz & Slie (25) reported that low concentrations of aureomycin inhibited the ability of cell free extracts of *E. coli* to reduce the nitro groups of chloroamphenicol and *p*-nitrobenzoic acid to the corresponding arylamines. On further analysis it was found that the reduction was mediated by DPN, and the aureomycin acted by interfering with the reduction of this carrier. This inhibition was readily reversed by the addition of an excess of Mn. The cytochrome-*c* reductase of *E. coli* has been partially purified and characterized by Brodie (26). The enzyme is a flavoprotein and very similar to the comparable enzyme of animal tissue. It is not definite, however, that this strain of *E. coli* has typical cytochrome-*c*, but it at least has a similar hemoprotein which is reduced by this enzyme when reduced DPN is the hydrogen donor. A unique situation in the oxidation of $DPNH_2$[2] by enzyme fractions from *Streptococcus faecalis* was encountered by Dolin (27). The organism contains no cytochrome-*c* but added cytochrome-*c* greatly accelerated the rate of $DPNH_2$ oxidation. The enzyme was FAD[2] dependent and was not inhibited by cyanide or azide. In the absence of added cytochrome-*c* the oxidation of $DPNH_2$ required $\frac{1}{2}$ mole of O_2/mole $DPNH_2$. There was no accumulation of peroxide and no catalase was present. The reaction was written as follows:

$$DPNH_2 + O_2 \rightarrow DPN + H_2O_2 \qquad 5.$$
$$DPNH_2 + H_2O_2 \rightarrow DPN + H_2O \qquad 6.$$
$$\text{Sum: } 2DPNH_2 + O_2 \rightarrow 2DPN + 2H_2O \qquad 7.$$

The peroxidation step could be made limiting by dilution of the enzyme and under these conditions peroxide accumulated. The enzyme responsible for the peroxidation was completely separated from the oxidase by ammonium sulfate fractionation so that aerobically there was no reaction in the absence of added H_2O_2.

Krebs (28) reported that it was possible to isolate several forms of yeast glyceraldehyde-3-phosphate dehydrogenase. The fact that the forms were separable both electrophoretically and in nucleic acid fractionation indicated that they were not in any kind of rapid equilibrium with one another. However, it seemed possible that the various forms were the result of changes incurred during drying and the fractionation procedure. Krebs, Rafter & Junge (29) noted that yeast protein 2, which has triose glyceraldehyde-3-

phosphate dehydrogenase activity, constituted over 5 per cent of the original protein of yeast. It was also found to be very similar to the protein isolated by Warburg & Christian and identified as triose phosphate dehydrogenase. In an extensive investigation of yeast glyceraldehyde-3-phosphate dehydrogenase Velick & Hayes (30) noted that phosphate binding could be completely suppressed by competing electrolytes without affecting the rate of oxidation. And in confirmation of earlier work it was concluded that the phosphate reacts by direct collision with bound and activated substrate. The enzyme could oxidase glyceraldehyde but had a very low affinity for this substrate. The crystalline enzyme was prepared from yeast by Velick & Udenfriend (31), who found that it contained two peptide chains terminated at the amino ends by valine. An enzyme-inhibitor complex was formed with two equivalents of *p*-chloromercuribenzoate (32). This inactive form was incapable of binding DPN.

Oxidative pathways.—In the past year considerable progress has been made towards the elucidation of the pathway of glucose oxidation in the obligate aerobic pseudomonads. Wood & Schwerdt (33) observed that cell free extracts of glucose-grown *P. fluorescens* contained the enzymes necessary for the oxidation of glucose, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, 6-phosphogluconate, ribose-5-phosphate, and gluconate. This would appear to confirm the conclusions of Entner & Stanier (34) that the nonphosphorylated scheme proposed by Stokes & Campbell (35) is not the pathway of glucose oxidation and that the production of 2-ketogluconate is the result of a side reaction. However, in confirmation of the observations of Stokes & Campbell it was been found by Wood & Schwerdt (33) that cell extracts of *P. fluorescens* oxidized glucose and gluconic acid quantitatively to 2-ketogluconate. In addition, these workers were able to show that the oxidation of glucose-6-phosphate and 6-phosphogluconate went well beyond the oxidation level of 2-ketogluconate. Subjection of the extract to an ammonium sulphate treatment resulted in the complete loss of ability to oxidize glucose-6-phosphate and 6-phosphogluconate whereas the oxidation of glucose and gluconate was unimpaired. It was, therefore, concluded that glucose and gluconic acid are oxidized by way of 2-ketogluconate whereas glucose-6-phosphate and 6-phosphogluconate were part of an independent pathway. In confirmation of their conclusions was the finding that the organism contained no hexokinase and therefore could not bring glucose into the usual phosphorylated pathway. The enzymes involved in the oxidation of glucose and gluconate by this organism are apparently quite different from the systems previously described for the oxidation of these two compounds in other types of tissue. There is no evidence that either oxidation is DPN or TPN linked nor is there evidence of a flavin carrier. However, cytochrome carriers are apparently involved (33). Confirmation of some of these observations has been reported by Claridge & Werkman (36). Further confirmation came from the work of Lemoigne, Blanchère & Aubert (37) who in addition found that the glucose oxidation

required Mg or Mn and that H_2O_2 accumulation cound not be detected. Similar systems for the oxidation of glucose and gluconate by *Neisseria winogradsky* were also described (38). Entner & Stanier (34) excluded 2-ketogluconate as an intermediate in a major pathway of oxidation of glucose or gluconate on the basis of two criteria. One was the low rate of 2-ketogluconate oxidation by intact cells and the other was that glucose and gluconate were apparently oxidized well beyond the 2-ketogluconate level of oxidation before the rate of oxidation was reduced to that of 2-ketogluconate. These do not seem to be adequate criteria for the evaluation of the status of an intermediate compound for it is known that intact cells of the pseudomonads oxidize 2-ketogluconate slowly even when grown with this compound as the sole carbon source (39). Moreover Entner & Stanier's second criticism of 2-ketogluconate as an intermediate applied equally well to gluconic acid, and yet they concluded that this compound was an intermediate in glucose oxidation. The fact that 2-ketogluconate was shown to accumulate in the medium in large quantities and that the rate of oxidation of glucose paralleled that of 2-ketogluconate after the first 60 min. could well be interpreted as indicating that all the glucose is oxidized by way of 2-ketogluconate but that the particular organism studied has an unusually weak system beyond 2-ketogluconate. In support of this contention is the fact that other workers have not been able to repeat these observations with related organisms (36, 39).

An interesting observation has been made by Sokatch & Gunsalus (40) to the effect that when *S. faecalis*, which has a conventional glycolytic scheme, was grown on a gluconic acid medium the culture was simultaneously adapted to 2-ketogluconate. During the fermentation of gluconic acid whole cells of the organism formed 0.5 *M* of CO_2, 1.5 *M* of lactate and at least one other acidic product. There is an interesting analogy between this organism and *P. aeruginosa* or *P. fluorescens* in that the pseudomonads have the enzymes necessary for the degradation of glucose-6-phosphate but have no hexokinase with which to bring glucose into the phosphorylated scheme. *S. faecalis* on the other hand apparently has a pathway through 2-ketogluconate but does not have a glucose oxidase to get it into this nonphosphorylated scheme.

The pathway of oxidation of glucose-6-phosphate and 6-phosphogluconate in *P. fluorescens* is apparently similar to that described for *Pseudomonas saccharophilia* by Entner & Doudoroff (41). Wood & Schwerdt (42) demonstrated that extracts of *P. fluorescens* oxidized 6-phosphogluconate in the presence of arsenite and hydrazine to virtually stoichiometric amounts of pyruvate and alkali labile phosphate. Only a slight amount of pentose was formed. Under the same conditions the breakdown of ribose-5-phosphate did not result in the accumulation of either pyruvate or alkali labile phosphate. The disappearance of ribose-5-phosphate, however, was coincident with the appearance of a compound tentatively identified as sedoheptulose.

Katznelson, Tanenbaum & Tatum (43) found that aged cell suspensions or cell free extracts of *Acetobacter melanogenum* quantitatively oxidized glucose, gluconic, or 2-ketogluconic acids to a common intermediate, thus supporting the view that 2-ketogluconate is part of a major pathway of glucose oxidation in many microorganisms. The intermediate compound in question was one atom of oxygen more oxidized than 2-ketogluconate and was postulated to be 2,5-diketogluconic acid. The difficulties in preparing a sample of the postulated compound by an independent method, the inability to form acceptable derivatives, the instability of the free acid or the salt even at room temperature, the failure to identify formate as a product of periodate oxidation, and the failure to demonstrate that the postulated intermediate could serve as a substrate for the organism make it impossible to conclusively decide that 2,5-diketogluconate is the intermediate under study. In contrast to these observations is the work of Foda & Vaughn (44) who concluded that *A. melanogenum* dissimilated maltose by way of glucose, gluconic acid, and 5-ketogluconic acid. 2-Ketogluconate could not be detected, and no evidence of more oxidized products was reported.

A mutant capable of utilizing glucose has been used by Klein (45) in an effort to elucidate the mechanism by which *Pseudomonas putrefaciens* dissimilates sucrose. Intact cells were permeable to both sucrose and glucose and were capable of oxidizing sucrose in the presence of glucose. By contrast, in cell extracts, glucose completely inhibited the phosphorolysis of sucrose. It was suggested that in intact cells the glucokinase is located at or near the cell surface with the consequent exclusion of free glucose from the interior of the cell where the sucrose phosphorylase may be active.

Scott & Cohen (46, 47) partially purified and characterized the glucose-6-phosphate dehydrogenase and the 6-phosphogluconate dehydrogenase of *E. coli* and on the basis of the relative activity of whole cells and cell extracts concluded that this pathway could account for at least 40 per cent of the glucose oxidized. The probability that this figure is not accurate is emphasized by the experiments of Sher & Mallette (48) on the lysine and arginine decarboxylases of *E. coli*. These workers found that extracts of the organism, obtained by lysing cells with the specific bacteriophage, exhibited four to nine times the total activity measurable in intact cells. It would appear that the potential activity within the cells may bear no obvious relation to the activity obtained with intact organisms. Cochrane, Peck & Harrison (49) noted that *Streptomyces coelicolor* had a strong hexose monophosphate shunt as evidenced by the rate of evolution of CO_2 from the C-1 position. Similar data for *Penicillium chrysogenum* were obtained by Heath & Koffler (50) and by DeFiebre & Knight (51).

Blumenthal, Lewis & Weinhouse (52) concluded that under aerobic conditions 80 per cent of the glucose utilized by *Torulopsis utilis* passed through the conventional Embden-Meyerhof system, the remainder being oxidized by the hexose monophosphate shunt. The glucose-6-phosphate

dehydrogenase of *Leuconostoc mesenteroides* has been partially purified and characterized and has been found to be nonspecific in its requirement for DPN or TPN [DeMoss, Gunsalus & Bard (53)].

Arnstein & Bentley (54, 55, 56) concluded that two species of *Aspergillus* converted glucose to kojic acid by a direct route not involving a splitting of the carbon chain. There was evidence, however, that two minor pathways probably involving the condensation of trioses was also operative. The condensation was not merely a reversal of glucose degradation for the labeling in glycogen formed from three-carbon compounds differed from that in kojic acid.

The statement of Krebs, Gurin & Eggelston (57) that "the tricarboxylic acid cycle supplies not primarily energy but intermediates" continues to attract attention and support. In addition recent studies on the utilization of three-carbon compounds in the synthesis of pentose, sedoheptulose, 6-phosphogluconate, and 2-phospho-4-hydroxy-4-carboxy-adipic acid indicate that a considerable portion of carbohydrate will not enter the cycle. Roberts *et al.* (58), on the basis of experimental evidence, calculated that during the oxidation of glucose by *E. coli* less than one-sixth of the carbon entered the tricarboxylic acid cycle.

Data, gathered on a variety of microorganisms, continue to support the concept of the almost universal distribution of the conventional tricarboxylic acid cycle. Eisenberg (59) carried out very careful and extensive experiments on *Rhodospirillum rubrum* and found that the complete cycle apparently was present. Although neither acetate nor acetyl phosphate was oxidized, acetate plus ATP[2] and oxaloacetate were converted to citrate. It has also been shown that *Propionibacterium pentosaceum* [Delwiche & Carson (60)], *S. coelicolor* [Cochrane & Peck (61)], *P. fluorescens* [Barrett & Kallio (62)], and *Ashbya gossypii* [Mickelson & Schuler (63)], possess most of the enzymes necessary for the functioning of the conventional cycle. Barban & Ajl (64) concluded that enzymes of *Escherichia freundi* reversibly converted α-ketoglutaric acid to isocitric acid and eventually to citrate. However, no data for the isocitrate→citrate conversion were presented.

Dagley & Dawes (65, 66) confirmed the earlier conclusion that the pathway of dissimilation of citrate in *Aerobacter aerogenes*, resulting in the production of oxaloacetate and acetate, is not merely the reversal of the condensing reaction. Grunberg-Manago & Gunsalus (67) demonstrated that sonic extracts of *E. coli* possess the enzymes necessary for the oxidation of the TCA[2] cycle intermediates. In addition they studied an adaptive system which splits citrate to oxaloacetate plus acetate. Englesberg *et al.* (68) found that the enzymes of the conventional cycle were under adaptive control in *Pasteurella pestis*. Gillespie & Gunsalus (69) partially purified a citridesmolase from *S. faecalis*, which splits citrate asymmetrically to oxaloacetate plus acetate. The reaction required manganese but not CoA[2] or phosphate.

A new pathway of citrate oxidation in yeast was described by Foulkes (70). The system was cyanide sensitive; no α- or β-keto acids could be de-

tected, and no volatile acids or lactate were formed. The data indicate that the new scheme operates at about the same rate and parallel to the conventional pathway in yeast. A factor required by the cell free extracts for the functioning of this new pathway was partially purified and studied by Foulkes (71). This citrate oxidation factor (COF) is weakly basic, contains no phosphorus, and is unstable in normal acid or alkali. Campbell, Smith & Eagles (72) found that extracts of *P. aeruginosa* dissimilated citrate or *cis*-aconitate anaerobically or aerobically with the production of succinate and glyoxylate. Attempts to detect the new reaction with isocitrate as substrate or to observe reversible formation of citrate from isocitrate failed. However, the organism has a strong ioscitric dehydrogenase and so it is probable that the conventional cycle is also operative. The dissimilation of the 6-carbon tricarboxylic acid to succinate plus glyoxylate makes an interesting addition to the list of important intermediates formed from the TCA cycle. In this case succinate can continue through the cycle while the glyoxylate is available for amino acid synthesis. The over-all picture of this series of reactions is that one mole of acetyl-CoA enters the cycle and is converted to glyoxylic acid and in addition oxaloacetate is converted to succinate. The energy produced during one revolution of the cycle should be only about 40 per cent of that obtained from the conventional scheme and so this cycle is even less important as an energy yielding mechanism than is the conventional one.

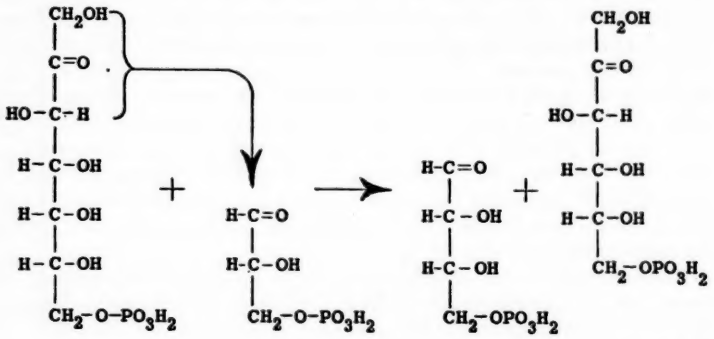
Umbreit (73) presented evidence that the product of the reaction between pyruvate and oxaloacetate in *E. coli* is the seven-carbon 2-phospho-4-hydroxy-4-carboxyadipic acid previously described by Rapoport & Wagner (74). The formation of this compound was inhibited by streptomycin. One explanation of why the compound has not been observed earlier was provided by Umbreit's finding that it was present in the discarded inorganic fraction when the conventional fractionation techniques were used.

Lindstrom (75) purified the α-ketoglutaric oxidase of *Azotobacter vinelandii* and found that DPT,[2] DPN, and Mg were necessary for maximum activity. Evidence was also obtained that succinyl-CoA was an intermediate in the oxidation of α-ketoglutarate. Lenhoff & Kaplan (76) noted a cytochrome peroxidase in *P. fluorescens*. Pinchot (77) observed that the oxidation of DPNH by extracts of *Alcaligenes faecalis* resulted in the generation of high energy phosphate. This phosphorylation was not inhibited by DNP.

A comparison of the aconitase, fumarase, and DPN linked isocitric dehydrogenase of normal and respiration deficient yeast has been carried out by Hirsch (78). He demonstrated that when grown anaerobically the mutant and the wild type contain essentially the same concentrations of the three enzymes. With aerobic growth conditions the wild type contained about five times as great a concentration of the enzymes as did the mutant. Haskins *et al.* (79) concluded that young cells of the poky strain of *Neurospora* contain a system which destroys the cytochromes. However, in older cultures large amounts of cytochrome-*c* accumulate. Ycas & Starr (80)

isolated a mutant of *S. cerevisiae* which had no respiratory activity and had no absorption bands corresponding to the cytochromes. The deficiency was apparently caused by a practically complete loss of glycine synthesizing ability for the addition of protoporphyrin IX or glycine resulted in the formation of catalase, cytochrome-*c*, and hematin. The occurrence of hemoglobin in freshly prepared extracts of yeast, *Neurospora crassa*, and *P. notatum* has been reported by Keilin (81) and by Keilin & Tissieres (82). However, no indication as to the function of this compound in microorganisms was obtained.

Horecker & Smyrniotis (83, 84) purified a transadolase from brewer's yeast. The enzyme catalyzed the transfer of carbons 1, 2, and 3 from sedo-



Equation 8.

heptulose to a suitable acceptor such as 3-phosphoglyceraldehyde (see equation 8). The nature of the residual tetrose was not established. Since only catalytic amounts of 3-phosphoglyceraldehyde were required, it was presumed that it must be formed from the tetrose. It was proposed that this mechanism could account for such reactions as the biological formation of glucose from galactose or ribose phosphate from xylose. Together with the oxidation of glucose by way of 6-phosphogluconate these reactions form a cyclic mechanism for the metabolism of glucose-6-phosphate.

Hochester & Watson (85) found that acetone-dried preparations of *Pseudomonas hydrophila* contained an enzyme which converted D-xylose to *d*-xylulose. Similar results were obtained by Mitsuhashi & Lampen with *Lactobacillus pentosus* (86). The latter workers also found that xylulose was phosphorylated much more rapidly than was xylose, and it was therefore suggested that the sequence of events in the degradation of xylose is

$$\text{xylose} \rightleftarrows \text{xylulose} \rightleftarrows \text{phosphorylated pentose} \qquad 9.$$

Lampen (87) has also shown that during the degradation of D-xylose or D-xylulose by *L. pentosus*, ribose-5-phosphate and possibly ribulose-5-phosphate are formed. No evidence for the formation of a xylose or xylulose

phosphate was obtained. In agreement with these observations was the work of Cohen (88) which indicated that *E. coli* formed an adaptive enzyme which converted D-arabinose to D-ribulose.

Racker, De LaHaba & Leder (89) isolated an enzyme from yeast and *E. coli* which, in the presence of DPT, Mg, and an "acceptor aldehyde," would convert ribulose-5-phosphate to D,3-phosphoglyceraldehyde plus an active 2-carbon ketol compound. With D,3-phosphoglyceraldehyde as an "acceptor aldehyde" the decarboxylation of hydroxypyruvate yielded ribulose-5-phosphate. Evidently no free glycolaldehyde was formed, but a ketol condensation involving "active glycolaldehyde" took place.

The discontinuity in the inhibitory effect of urethane on yeast respiration has been found by Lamanna & Campbell (90) to coincide with irregularities in the surface tension of a series of dilutions of the inhibitor. In confirmation of this conclusion was the observation that cetyl pyridinium chloride, which is strongly surface active, showed a similar but much more pronounced discontinuity when oxygen uptake data were plotted against the concentration of the inhibitor. Again the break in the oxygen uptake curve and the break in the surface tension curve occurred at the same concentration of inhibitor. These observations indicate that earlier data which had been interpreted as indicative of the presence of two pathways of metabolism in yeast may be better explained on the basis of these irregularities in the physical characteristics of a series of dilutions of the inhibitor.

Posternak & Reymond (91) extended Chargaff's work on the rules governing the oxidation of cyclitols by *Acetobacter suboxydans* to the cyclohexanetetrols, cyclohexanetriols, and lower cyclic alcohols. They concluded that the oxidation of tetrols and lower alcohols did not seem to follow either Bertrand's or Chargraff's rules. Anderson *et al.* (92) were able to show that some of the apparent exceptions to Chargraff's rules were caused by the activity of a mixture of oxidases capable of attacking different positions in the molecule.

Cell extracts of several mycobacteria grown on a glycerol medium have been shown by Hunter (93) to utilize glycerol by way of the triose steps of the Embden-Meyerhof pathway. The extracts contained glycerolphosphate dehydrogenase, accumulated pyruvate in the presence of arsenite when glycerol was substrate, and converted 3-phosphoglyceric acid to phosphoenolpyruvic acid. Asnis & Brodie (94) partially purified glycerol dehydrogenase from *E. coli*. The enzyme was DPN linked, had a pH optimum of 10.0, did not require phosphate, and resulted in the formation of dihydroxyacetone. On the basis of experiments employing the technique of simultaneous adaptation Magasanik *et al.* (95) concluded that phosphorylated intermediates were not involved in the dissimilation of glycerol by a strain of *A. aerogenes*. It should be remembered, however, that this technique may not be applicable when intact cells and phosphorylated substrates are used.

Fermentation.—According to the observations of Neish (96), the anaerobic dissimilation of glucose by *Bacillus subtilis* occurs almost exclusively by the Embden-Meyerhof pathway. On the basis of the fact that 6 per cent of

the carbon-1 glucose appeared as CO_2 it was suggested that a minor alternate pathway was also operative. In the light of our present knowledge of pathways of glucose dissimilation it would appear that this minor pathway would be similar to the scheme operative in the heterofermentative lactic acid bacteria. The amount of CO_2 fixed was much higher at an alkaline pH indicating that the bicarbonate ion was metabolized more readily than undissociated carbonic acid. Using glucose-1-C^{14} and glucose-3,4-C^{14} Gibbs & Gastel (97) found that *Rhizopus* fermented glucose to CO_2, ethyl alcohol, and lactic acid. The distribution of labelled carbon could be completely accounted for by the Embden-Meyerhof system. Under aerobic conditions the pathway to lactic acid predominated, and there was no evidence that any of the lactate came from a four-carbon acid.

Rutter & Hansen (98) noted that a strain of *Lactobacillus bulgaricus* could grow on lactose but could not grow with glucose or galactose as sole carbon sources. In spite of this the two hexoses were readily fermented. Resting cells could also ferment α-galactose-1-phosphate but not β-galactose-1-phosphate, α- or β-glucose-1-phosphate, or glucose-6-phosphate. The organism has been shown to contain galactokinase, galactowaldenase, and phosphoglucomustase and so is capable of converting galactose to glucose-6-phosphate (99).

Uridine diphosphate acetylglucosamine has been isolated by Cabib, Leloir & Cardini (100) from baker's yeast. The data support the suggestion that uridine-5′-pyrophosphate and acetylglucosamine are joined through a glycosidic link. It would appear likely that this compound would play an active role in the metabolism of hexose amines. However, preliminary experiments on this aspect of the problem yielded negative results.

An explanation for the disappearance of UDPG[2] in the presence of yeast extract and glucose-6-phosphate has been proposed by Leloir & Cabib (101). They found that trehalose phosphate was formed coincident with UDPG disappearance. It was suggested that the reaction could be written as follows:

$$\text{UDPG} + \text{glucose-6-phosphate} \rightarrow \text{UDP} + \text{trehalose phosphate} \qquad 10.$$

During the purification process the galactokinase content of extracts of *S. fragilis* was found to be paralleled by the content of the enzyme which phosphorylates galactosamine. As a result Cardini & Leloir (102) concluded that they were one and the same. In accord with this suggestion was the evidence that galactosamine-l-phosphate was the product of the phosphorylation of galactosamine.

Higher carbohydrates.—Extracts of a series of molds, including *Penicillium spinulosum* and *Aspergillus niger*, were shown by Bealing and Bacon (103) to liberate glucose and form four fructose-containing oligosaccharides during the breakdown of sucrose. The compounds were not formed when monosaccharides were used as substrates. It was concluded that the reaction was a transfer of fructose residues to suitable acceptors by a fructose

furanosidase. It was suggested that the same enzyme could use water as acceptor at a very slow rate with the resultant formation of free fructose. At least in the case of *P. spinulosum* this enzyme was considered to be the only one available for the dissimilation of sucrose. The general occurrence of this type of reaction was emphasized by the work of Pan, Nicholson & Kolachov (104). They reported that, in the presence of filtrates of *A. niger*, isomaltose, maltose, and sucrose underwent transglycosidation resulting in the formation of new trisaccharides. No evidence for the participation of phosphate could be found. The occurrence of similar activity was found in commercial or laboratory fungal amylase preparations, in *B. subtilis* preparations, and in barley malt (105). Jermyn & Thomas (106) reported that the *p*-nitrophenyl-β-glucosidase of *Aspergillus oryzea* was capable of transferring a glucose residue to methanol, ethanol, or *n*-butanol. In an investigation of mold invertase Bealing (107) found that all experimental data could be explained on the basis of the enzyme being a fructosidase and not a glucosaccharase as is commonly reported in the literature. Koepsell *et al.* (108) have carried out an extensive investigation of the activities of the dextransucrase of *L. mesenteroides*. With sucrose as substrate it was found that isomaltose, maltose, α-methylglucoside, glucose, and to a lesser extent a number of other sugars acted as efficient alternate glucosyl acceptors. In addition, they could initiate chain formation. In contrast to normal dextran synthesis the process in the presence of the alternate acceptors led to the extensive formation of oligosaccharides and the simultaneous growth of many dextran chains. The oligosaccharides formed were apparently the result of successive additions of glucose by α-1, 6-glucopyranosidic linkages on the alternate acceptor. A new fructose containing disaccharide, leucrose, was formed in all instances. Studies with this same enzyme by Hehre (109) revealed that the addition of low molecular weight dextrans profoundly affected the synthesis. With glucose, maltose, or panose as acceptors normal dextran was synthesized. Whereas, in the presence of any one of four low molecular weight dextrans greatly increased amounts of oligosaccharide were produced.

Organic Acids

Pyruvic.—Since several excellent reviews (110, 111) on the subject have appeared in the past year and since little new information is available the very important problem of the metabolism of pyruvate and related compounds will be reviewed only very briefly.

Using vacuum dried cells of *S. faecalis* and the ferricyanide assay system Dolin (112) showed that the oxidation of diacetyl required lipoic acid whereas the oxidation of acetaldehyde did not. Moreover, soluble extracts of these cells would oxidize diacetyl but not acetaldehyde, showing that free acetaldehyde was not an intermediate in the oxidation. The activity of these extracts was completely cocarboxylase dependent. Dolin emphasized that lipoic acid has a general function in reactions involving the split of the α-dicarbonyl grouping to active acetaldehyde followed by dehydrogenation of the active

acetaldehyde to form the acyl compound. In confirmation of the prevailing opinions on pyruvate degradation Strauss (113) has shown that although an acetate requiring mutant of *N. crassa* could not oxidize pyruvate, it could convert it to acetylmethylcarbinol. Experiments with *Tetrahymena* extracts by Seaman (114) indicate that α-lipoic acid is not necessary for the decarboxylation of pyruvate nor for the reversal of the reaction. The addition of α-lipoic acid actually eliminated the carboxylation reaction by making possible the oxidation of the two-carbon fragment. It was also concluded that reduction of ferricyanide by this organism during pyruvate oxidation was not mediated by α-lipoic acid. The removal of α-lipoic acid by alumina treatment of extracts of *Tetrahymena* resulted in a greatly diminished rate of acyl formation (115). Using a purified pyruvic oxidase from a mutant strain of *E. coli*, Reed & DeBusk (116, 117) have confirmed and extended their earlier conclusion that the amide of α-lipoic acid and DPT, which they call lipothiamide pyrophosphate, is essential for the decarboxylation of pyruvate. The decarboxylation reaction was inhibited by the addition of DPT. In addition, these workers concluded that, when ferricyanide was the electron acceptor, the oxidation did not require the lipoic acid derivative but did require DPT. Since these results are not in agreement with those from other laboratories, it is obvious that more work is required before any final conclusions can be reached with respect to the sequence of events and the role of cofactors in the degradation of pyruvate. Hager & Gunsalus (118) purified lipoic acid dehydrogenase 200-fold and confirmed the earlier suggestion that fraction B of *E. coli* is a dehydrogenase, common to keto acid systems, that links lipoic acid to DPN.

In a combined effort the Illinois, Texas, and Eli Lilly Research groups have isolated, characterized, and synthesized α-lipoic acid (119, 120, 121). The isolation represented a 300,000 to 600,000-fold concentration. It was concluded that α-lipoic acid is the cyclic disulphide derived from 4,8-, 5,8- or 6,8-dimercapto-*n*-caprylic acid. Treatment of α-lipoic acid with thiols, followed by oxidation to disulphides with iodine, resulted in the formation of a series of new ninhydrin reacting compounds possessing biological activity corresponding to that of α-lipoic acid. It is suggested that such compounds will be naturally occurring. It was also shown that β-lipoic acid is an oxidized form of α-lipoic acid, probably a sulfoxide. Seaman (122) found that *Lactobacillus casei* would convert protogen B (β-lipoic acid) to protogen A (α-lipoic acid).

Continuing his work on the mechanism by which propionate interferes with the metabolism of microorganisms, Hill (123) found that riboflavin, in addition to α-lipoic acid, was necessary for reversing propionate inhibition. The riboflavin presumably was necessary to complete the system for the formation of acetate from pyruvate and the acetate so formed overcame the inhibition. L-Lyxoflavin, which competitively inhibits riboflavin, prevented the action of riboflavin in the reversal of propionate inhibition.

Conway & O'Malley (124) concluded that in the decarboxylation of keto

acids by yeast carboxylase, CO_2 and carbonic acid are both primary products, but one or the other may predominate depending on conditions.

Propionic.—Several recent contributions have opened fresh avenues of investigation into the mechanism of formation of propionic acid from succinate. Delwiche, Phares & Carson (125) obtained evidence that cell-free extracts of *Propionibacterium pentosaceum* converted succinic, propionic, and acetic acids to the corresponding CoA derivatives. In a reaction requiring CoA and ATP it was shown that the cell extracts converted succinate-1-C^{14} into propionate-1-C^{14} (30 per cent yield of C^{14}) and $C^{14}O_2$ (1.5 per cent yield of C^{14}). With propionate-2-C^{14} and $C^{14}O_2$ as substrates, in the presence of unlabelled succinate, the incorporation of the propionate into succinate-1,2-C^{14} was demonstrated to be 60 times as great as the $C^{14}O_2$ incorporation. In a later discussion of the project Phares, Carson & Delwiche (126) reported that the ratio of labelled propionate to labelled CO_2 formed from succinate-1,4-C^{14} was 10:1. In the presence of CO_2 and malate and with malonate to inhibit the possible formation of malate by way of fumarate, cell-free extracts converted succinate-1,2-C^{14} to malate having five times as high a concentration of C^{14} in the carboxyl carbons as in the noncarboxyl carbons. It was also shown that the γ-carbon of succinyl-CoA was the one which by recombination with a three carbon derivative of propionate offsets the expected 1:1 ratio of propionate to CO_2. These workers concluded that this one carbon fragment from the γ-carbon of succinyl-CoA may combine reversibly with propionate to give succinate, or, may form CO_2, or be incorporated into malate carboxyls without going through fumarate or CO_2. This postulated activated one-carbon compound could well be the same as that envisaged by Utter (127) in his efforts to explain the mechanism of β-carboxylation of pyruvate. Whiteley (128, 129, 130) has shown that extracts of *Micrococcus lactilyticus* require ATP and CoA in the presence of cocarboxylase for the decarboxylation of succinate to propionate. Succinyl-CoA was shown to be rapidly decarboxylated by extracts which were depleted of ATP. Propionyl-CoA was found to be formed during the decarboxylation of succinyl-CoA, and it has been postulated that the sequence of reactions as seen in Figure 2 occurs:

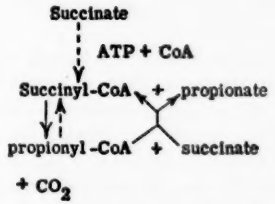


FIG. 2. Propionate formation from Succinate.

In accordance with this scheme extracts of the organism decarboxylate large quantities of succinate in the presence of only catalytic amounts of ATP.

Propionyl-CoA reacts with succinate in the presence of the extracts yielding succinyl-CoA+propionate. In this way another molecule of succinate is activated for the decarboxylation reaction. Whiteley draws a parallel between the formation of acetoacetic acid from two moles of acetyl-CoA and the formation of succinate from propionyl-CoA plus the activated one-carbon compound; this view is supported by the data of Phares *et al.* (126).

The turnover of CO_2 during the fermentation of three, four, five, and six-carbon compounds by propionic acid bacteria was determined by Wood & Leaver (131). No definite conclusions were drawn, but the data indicated that there was a mechanism for the formation of propionate by direct reduction. In confirmation of the other contributions in this field it was concluded that a C_1 compound other than CO_2 took part in the fixation reaction.

Using *P. fluorescens* Hayaishi (132) has obtained data indicating that the mechanism of decarboxylation of malonate may be very similar to that of succinate. CoA and ATP participated in the reaction, and two hydroxamic acid derivatives were separated chromatographically. Neither co-carboxylase nor Mg^{++} appeared to influence the reaction, however.

C_1 and C_2 compounds.—In a continuation of their work on the factor in yeast extract which stimulates formate breakdown, Lichstein & Boyd (133) have shown that yeast extract did not stimulate formic dehydrogenase with methylene blue as hydrogen acceptor. However, stimulation was evident with O_2 as the hydrogen acceptor and so it would appear that it is the electron transport system of the organism which is affected. In contrast oleic acid stimulated the reaction in the presence of methylene blue or oxygen.

Takamiya (134, 135) continued his studies on the formic dehydrogenase of *E. coli* making use of the competetive inhibitor hypophosphorous acid. Data on the kinetics of the reaction and the quantity of enzymes within the cell were reported.

Continuing his studies on *Aerobacillus polymyxa* Crewther (136) established the growth conditions necessary for the production of resting cells having strong hydrogenlyase activity. A medium having a high iron content was shown to be desirable, and it appeared that the iron functioned by forming or stimulating the formation of a protective gel around the cell. The addition of acid washed kaolin or to a lesser extent $CaCO_3$ had a similar protective effect.

Jefferson & Foster (137) have reported that formate or an active derivative is an intermediate in the metabolism of a strain of *Rhizopus*. The C^{14}-formate was converted to β-labelled pyruvate. This introduces a type of pyruvate synthesis not previously reported in fungi. Formaldehyde seemed to be used equally as well as formate in the fixation reaction. The disproportionate loss of hydrogenlyase activity as a result of diluting extracts of *E. coli* and the subsequent sparking of this enzyme reaction by the addition of extracts of *Clostridium butylicum* plus pyruvate has been explored further by Gest, Swim & Barkulis (138). It was found that the enzyme extract could not be replaced by yeast carboxylase nor by the acetoin forming system of *A. aero-*

genes. Diacetyl, however, could replace pyruvate in the sparking system. On the basis of additional experiments which showed that after an induction period, catalytic amounts of acetyl phosphate also stimulated the decomposition of formate Barkulis & Gest (139) concluded that an active C-2 compound was the sparking agent in the hydrogenlyase reaction. None of the common coenzymes tested was the sparking agent and acetyl-CoA actually inhibited the sparking reaction. Two alternate schemes compatible with the results were presented as schemes A and B (See Fig. 3).

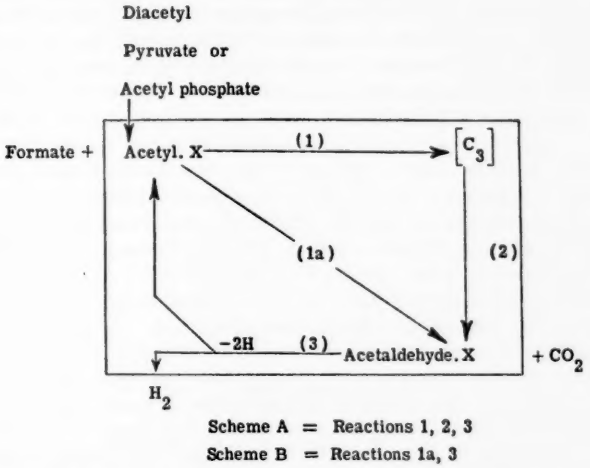


FIG. 3. Role of C^2 derivatives in the decomposition of Formate.

According to scheme A (Reactions 1, 2, 3) an intermediate C-3 compound is formed which undergoes decarboxylation yielding CO_2 and an acetaldehyde complex. The latter is then oxidized, thus regenerating acetyl.X; the electrons are transferred to hydrogenase with the ultimate formation of H_2.

In scheme B (Reactions 1a, 3) formate is dehydrogenated directly with acetyl.X serving as hydrogen acceptor. Molecular H_2 is subsequently formed and acetyl.X regenerated as in scheme A. It was suggested that acceptors other than hydrogenase may act as oxidants for the acetaldehyde derivative in which case molecular H_2 would not be produced.

Acceptance of scheme A implies that formation of H_2 and CO_2 from pyruvate by *E. coli* and *C. butylicum* occurs through a common pathway. Thus both organisms would activate pyruvate to C-3 and thence through reactions 2 and 3 to acetate, H_2, and CO_2. The inability of *C. butylicum* to convert formate to H_2 and CO_2 could be explained as attributable to absence of reaction 1. Reversal of reaction 1 would be visualized as one of the initial stages in the classical phosphoroclastic split of pyruvate to acetate and formate.

The nature of the C-2 derivatives in the above schemes is not specified. Conceivably, any of the currently postulated C_2-coenzyme compounds (C_2-lipoic, C_2-lipothiamide, or C_2-cocarboxylase) may be involved. Crewther (140) also noted the loss of hydrogenlyase activity on dilution but attributed the inactivation to a destruction of the enzyme or components of the enzyme system.

Additional evidence has been obtained that the acuumulation of 4-amino-5-imidazolecarboxamide in *E. coli* in the presence of sulfathiazole is a result of the inhibition of formate metabolism. Stewart & Sevag (141) found that although sulfathiazole inhibited the utilization of glucose by 50 per cent the evolution of H_2 was actually increased. When formate was the substrate evolution of H_2 was increased in the presence of sulfathiazole to as much as 200 per cent of the control. The conclusion was that the sulfathiazole stepped up the degradation of formate and therefore interfered with the availability of this compound for synthesis. In confirmation of this suggestion was the additional observation that a strain which was devoid of hydrogenlyase failed to accumulate 4-amino-5-imidazolecarboxamide even during sulfathiazole bacteriostasis. These observations are in line with the known facts that formate is the precursor of carbon-2 in purines and that 4-amino-5-imidazolecarboxamide lacks carbon-2 for the ring closure.

Working with a purified yeast enzyme and radioactive inorganic pyrophosphate Jones *et al.* (142) further clarified the reactions occurring during the conversion of acetate to acetyl-CoA. It was found that, in the presence of the yeast enzyme, inorganic pyrophosphate exchanged rapidly with the pyrophosphoryl group in ATP. This indicated the following reaction:

$$\text{Enz} + \text{Ad} - \text{P} \sim \text{PP} \rightleftarrows \text{Enz} \sim \text{P} - \text{Ad} + \text{PP} \qquad 11.$$

CoA inhibited this exchange reaction proportional to concentration. This suggested that $AMP^2 \sim$enzyme subsequently exchanged AMP for CoA. Therefore the presence of CoA decreased the concentration of AMP$\sim$enzyme and thereby slowed down the rate of exchange with pyrophosphate. The additional sequence of events was therefore proposed.

$$\text{Enz} \sim \text{P} - \text{Ad} + \text{HSCoA} \rightleftarrows \text{Enz} \sim \text{SCoA} + \text{Ad} - \text{P} \qquad 12.$$

$$\text{Enz} \sim \text{SCoA} + \text{CH}_3\text{COOH} \rightleftarrows \text{Enz} + \text{CH}_3 \cdot \text{CO} \sim \text{SCoA} \qquad 13.$$

A TPN specific aldehyde dehydrogenase has been purified from baker's yeast by Seegmiller (143). Acetaldehyde, glycolaldehyde, propionaldehyde, and formaldehyde served as substrates while the enzyme was activated by Ca, Mg, Ba, and Mn. Acetic acid was found to be the product of acetaldehyde oxidation. However, there was no evidence of CoA participation.

Cell free extracts of *Clostridium kluyveri* have been shown by Stadtman (144) to contain an enzyme system, coenzyme A transphorase. This enzyme catalyzed CoA transfer from acetyl-CoA to other fatty acids. The sequence of events can be written as follows:

$$\text{Acetyl} \sim P^2 + \text{CoA} \rightleftarrows \text{acetyl} \sim \text{CoA} + P_i^2 \quad 14.$$

$$\text{Acetyl} \sim \text{CoA} + \text{propionate} \rightleftarrows \text{propionyl} \sim \text{CoA} + \text{acetate} \quad 15.$$

$$\text{Propionyl} \sim \text{CoA} + P_i \rightleftarrows \text{propionyl} \sim P + \text{CoA} \quad 16.$$

Aromatic acids.—On the basis of adaptation studies Gale (145) concluded that a variety of mycobacteria oxidize benzoate by way of catechol but not by way of β-ketoadipic acid. This conclusion was based on the finding that intact cells or extracts from cells adapted to benzoate were also adapted to catechol but not to β-ketoadipic. Sistrom & Stanier (146) dismiss Gale's work by stating that his results were obtained exclusively with intact cells. Nevertheless Gale had used cell extracts produced by grinding and by ultrasonic vibrations. Sistrom & Stanier reinvestigated this work with cell extracts of *M. butyricum* and confirmed Gale's data. However, in addition, they found that extracts from cells adapted to benzoate oxidized catechol with the quantitative accumulation of β-ketoadipic, and they concluded that this latter compound is therefore an intermediate in the oxidation of catechol.

Gunsalus, Gunsalus & Stanier (147, 148, 149), in a series of careful experiments, have separated and studied the enzymes of *P. fluorescens* A.3.12 which dissimilate mandelic acid to benzoate. The pathway: mandelic acid→benzoylformic acid→benzaldehyde→benzoic acid, which had been proposed on the basis of experiments with whole cells was confirmed by their work. The organism was found to contain a substrate induced racemase active on D(−) or L(+) mandelic acid. The mandelic dehydrogenase was active only on the L(+) configuration, and no cofactor requirement could be detected. The benzoylformate carboxylase required DPT but not Mg and appeared to be specific for benzoylformate. Benzaldehyde was dehydrogenated by either one of two highly specific dehydrogenases. One of these required DPN for activity while the other required TPN. Employing simultaneous adaptation and also isolation techniques Gunter (150) obtained evidence that *P. fluorescens* A.3.12 oxidizes *p*-hydroxymandelic by way of *p*-hydroxybenzaldehyde, *p*-hydroxybenzoate, and *p*-hydroxyprotocatechuate. *p*-Hydroxybenzoylformate was tentatively identified as a member of this aromatic pathway. The oxidation of *p*-hydroxymandelate apparently parallels that of mandelate.

On the basis of results obtained with simultaneous adaptation studies Sakamoto, Uchida & Ichihara (151, 152) concluded that an unidentified gram negative rod oxidized indole by way of isatin→formylanthranilic acid→salicylic acid→catechol. Vitamin B_{12}, folic acid, glutathione, and adenylic acid appeared to be necessary for activity of the indole oxidizing system. Additional work from their laboratory (153, 154) indicated that *E. coli* produced phenol from tyrosine by a direct anaerobic fission and not by a stepwise reaction. Evidence that pyridoxal phosphate was the coenzyme of this phenol forming system was presented. On the basis of adaptive patterns and isolation techniques Walker & Wiltshire (155) concluded that a gram negative, aerobic soil bacterium oxidizes naphthalene by a pathway including salicylic acid, catechol, and D-*trans*-1,2-dihydro-1,2-dihydroxynapthalene.

Davis & Mingioli (156) and Salamon & Davis (157) isolated a compound, formulated as 5-dehydro-shikimic acid, as a precursor of shikimic acid in the metabolism of *E. coli*. Weiss, Davis & Mingioli (158) isolated and identified 5-dehydroquinic acid as a precursor to 5-dehydroshikimic acid, shikimic acid, and therefore to the aromatic compounds tyrosine, phenylalanine, tryptophan, *p*-aminobenzoic acid, and *p*-hydroxybenzoic acid.

Miscellaneous.—A FMN requirement for luminescence of *Achromobacter fischeri* has been reported by McElroy *et al.* (159). Cormier & Strehler (160, 161) have obtained evidence that the kidney cortex factor (KCF), which is required for luminescence with extracts of *A. fischeri*, is palmitaldehyde. The aldehyde gave a 5 to 10-fold increase in luminescence at a concentration of 5×10^{-7} gm./ml. Other long chain fatty aldehydes were also active but shorter homologues were not.

Nitrogen Compounds

Inorganic nitrogen.—Using cell free extracts of a halotolerant aerobic bacillus Taniguchi *et al.* (162) studied the stepwise conversion of NO_3 to NH_3. Using leucomethylene blue as a hydrogen donor, they demonstrated that the extracts contained a cyanide sensitive nitratase, a strong nitritase, and a hydroxylamine reductase. Marshall *et al.* (163) concluded that, in the case of *Pseudomonas denitrificans*, ammonia is an intermediate in the reduction of nitrate to cellular nitrogen. However, the conversion of nitrate to gaseous nitrogen apparently did not involve ammonia. Denitrification apparently was achieved in strongly aerated cultures. In support of the theory that nitramide is an intermediate in the reduction of nitrate to nitrite Allan & Najjar (164) found that extracts of *P. stutzeri* and *B. subtilis* converted nitramide to nitrogen and aged or diluted extracts converted nitramide to nitrite and hydroxylamine. Since the latter two compounds are generally recognized as intermediates in nitrate oxidation, these data indicate that nitramide is a possible intermediate. It was also found that lysates of a strain of *B. subtilis* converted at least some nitrite to N_2O (165). Nicholas, Nason & McElroy (166) demonstrated that molybdenum was necessary for the synthesis or for the activity of the nitratase of strains of *Neurospora* or *Aspergillus*. The TPN linked nitratase of *Neurospora* has been shown by Nason & Evans (167) to be a flavoprotein with FAD as the prosthetic group.

By the use of selective inhibitors Hofman & Lees (168) were able to demstrate the intermediate formation of hydroxylamine during the conversion of ammonia to nitrite. Allylthiourea was found to selectively inhibit the oxidation of ammonia thus indicating the need of a heavy metal ion for this step. Hydrazine interfered with the breakdown of hydroxylamine and thereby allowed its detection during the oxidation of ammonia.

Newton, Wilson & Burris (169) demonstrated that during short time exposures of *Azotobacter* to N_2^{15} in the presence of ammonia the ammonia recovered contained the highest concentration of N^{15}, greatly surpassing the isotope concentration of any other cellular fraction isolated. These experi-

ments were carried out under conditions permitting little or no formation of ammonia as a product of deamination. These data confirm, in a direct fashion, earlier observations which indicated that ammonia was an intermediate in the pathway of nitrogen fixation. Hamilton, Magee & Mortenson (170) have presented strong evidence that several strains of *A. aerogenes* fix small amounts of N_2 and so it would appear that nitrogen fixation may be a very widespread phenomenon.

Using cell free extracts of *Azotobacter* Green & Wilson (171) confirmed their earlier conclusions that hydrogenase has a particular significance in nitrogen fixing organisms. This confirmation was obtained from experiments in which the hydrogenase content of cell free extracts was shown to be appreciably higher when the cells had been grown on N_2 than when grown on NH_4-N, NO_3-N, or casein hydrolysate. An improved assay was introduced, but even this is valid only if hydrogenase is the limiting factor in a methylene blue reduction. Hyndman, Burris & Wilson (172) found that hydrogenase from *A. vinelandii* was associated with the particulate fraction. In agreement with other workers it was considered to be an iron enzyme but not necessarily a porphyrin although it was felt that it at least functioned in conjunction with iron porphyin enzymes. The oxidation of H_2 by this enzyme, with the formation of water, resulted in the production of high energy phosphate. Hydrogenase activity was not affected by additions of DPN, TPN, FAD, or FMN.

Amino acids.—Rudman & Meister (173) fractionated the proteins of *E. coli* and were able to obtain two separate transaminases. One catalyzed reactions involving phenylalanine, tyrosine, tryptophan, and aspartate while the second was active on isoleucine, valine, leucine, norvaline, and norleucine. The amino acids of each group transaminated with each other but not at all or only relatively slowly with those of the other group. Althenbern & Housewright (174) found that *Brucella abortus* also had several transaminases and that these did not necessarily involve glutamate. β-Alanine and γ-aminobutyric acid, the decarboxylation product of glutamic acid, have been shown by Roberts, Ayengar & Posner (175) to serve as the transaminating agents for α-ketoglutarate in *Aspergillus fumigatus*. The glutamate-aspartate transaminase of a halophylic *Pseudomonas* was partially resolved by Robinson & Katznelson (176). The enzyme had optimum activity in 4 to 8 per cent NaCl and was inactivated irreversibly if stored in the absence of NaCl.

Mamelak & Quastel (177) have reinvestigated the long dormant problem of the mechanism of the Stickland reaction. Pyruvate was shown to be an intermediate in the proline-alanine interaction and DPN acted as carrier. Evidence for the formation of acetyl-CoA during this anaerobic reaction was also obtained.

Katz, Lieberman & Barker (178) found that, in the presence of cyanide or azide, dried cells of *C. kluyveri* would catalyze the acetylation of glycine by acetyl phosphate. The enzyme was capable of acetylating a wide range of amines, amino acids, and peptides. It was also shown that propionyl phos-

phate would react at about the same rate as the acetyl derivative (179). Butyryl phosphate did not react similarly to the lower acyl phosphates. However butyrate, isobutyrate, *n*-valerate, and *n*-caproate could be activated by acetyl or propionyl phosphate. It was assumed that the acyl-CoA derivative was the active form.

A somewhat similar series of reactions has been proposed by Stadtman & White (180) to account for acetyl transfer by imidazole containing compounds. It was found that in the absence of inorganic phosphate imidazole was acetylated with the formation of a labile compound tentatively identified as N-acetylimidazole. In aqueous solution this compound would react with amino acids, alcohols, inorganic phosphate, and sulfhydryl compounds to yield the corresponding acetyl derivative. The sequence of events was pictured as follows:

$$\text{Acetyl-P} + \text{CoA} \xleftarrow{\text{(phosphotransacetylase)}} \text{acetyl-CoA} + P_i \qquad 17.$$

$$\text{acetyl-CoA} + \text{imidazole} \xleftarrow{\text{(imidazole acetylase)}} \text{acetylimidazole} + \text{CoA} \qquad 18.$$

$$\text{acetylimidazole} + \text{cysteine} \xrightarrow{\text{(nonenzymatic)}} \text{imidazole} + \text{N-acetylcysteine} \qquad 19.$$

$$\text{Sum: Acetyl-P} + \text{cysteine} \rightarrow \text{N-acetyl cysteine} \qquad 20.$$

A reaction similar to that described by Jones *et al.* (142) for the formation of acetyl CoA from acetate, ATP, and CoA has been suggested by Maas & Novelli (181) to explain the formation of pantothenic acid from alanine and pantoic acid.

$$\beta\text{-alanine} + \text{pantoic acid} + \text{ATP} \rightarrow \text{pantothenic acid} + \text{AMP} + PP_i \qquad 21.$$

The pyrophosphate split of ATP was an essential part of the reaction and ATP could not be replaced by ADP[2]. It seems probable that the formation of an enzyme~AMP compound is the initial step in the sequence of reactions.

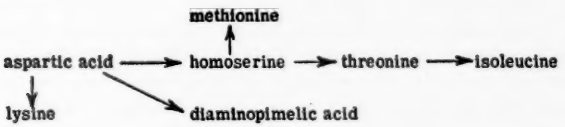
Snoke (182) prepared a highly purified enzyme from brewer's yeast which would synthesize glutathione from L-γ-glutamyl-L-cysteine plus glycine and ATP. An equivalent amount of inorganic phosphate was formed. It was concluded that the initial step was the phosphorylation of the enzyme by ATP and that the enzyme which catalyzed this reaction also catalyzed the next reaction, the synthesis of glutathione. Extracts of *E. coli* have been shown to synthesize glutathione from glutamic acid, cysteine, and glycine in the presence of K_2HPO_4, hexose-diphosphate, ATP, DPN, and Mg (183). Lester (184) found that a particulate system, obtained by subjecting *Micrococcus lysodeikticus* to the action of lysozyme, could incorporate leucine into protein. It was therefore concluded that bacteria contain organized aggregates which have special properties such as amino acid incorporation.

The presence of a decarboxylase for allo-β-hydroxy, DL-glutamic acid, in *E. coli*, was demonstrated by Umbreit & Heneage (185). A pyridoxal phos-

phate dependency could be shown after treatment of the preparation with an acid phosphatase for 4 hr.

Studies with the L-serine dehydrase of *Neurospora* by Yanofsky & Reissig (186) revealed that, in contrast to the comparable enzyme from *E. coli*, it had an absolute requirement for pyridoxal phosphate. The growth of *E. coli* on a glucose-inorganic salts medium in the presence of labelled oxaloacetate, aspartate, or formate, was found by Delluva (187) to result in the formation of labelled threonine but not of labelled serine. It would therefore appear that serine was formed exclusively from glucose whereas at least some of the threonine came from the four-carbon acids.

Using auxotrophic mutants Hirsch & Cohen (188) showed that *E. coli* contained an enzyme system capable of converting aspartic acid to threonine and that L-homoserine was an intermediate in the reaction. Black & Gray (189) purified an enzyme from yeast which would phosphorylate L-aspartic acid in the presence of ATP. The product of the reaction was β-aspartyl phosphate. In a continuation of this work Black & Wright (190) reported that a yeast preparation, which was TPN specific, could reduce β-aspartyl phosphate to homoserine. Presumably these are steps in the formation of threonine. Abelson *et al.* (191), on the basis of results obtained with the isotope competition technique, concluded that aspartic acid was involved in the following reactions:



These workers also used glutamate as substrate and confirmed the observations of Vogel's group with respect to ornithine and proline synthesis.

Using mutants of *E. coli* Vogel (192) has been able to greatly clarify the mechanisms for the formation of ornithine from glutamic acid. Maas, Novelli & Lipmann (193) found that extracts of *E. coli* could acetylate glutamic as well as other amino acids. The reaction required CoA and in contrast to the experiments of Katz *et al.* (178) cyanide was not required. Vogel, Abelson & Bolton (194) confirmed that this acetylation was an intermediate step in the formation of ornithine from glutamate. They also found evidence of a pathway of proline synthesis involving N-acetyl glutamic acid but not involving glutamate. This indicated the presence of a minor pathway other than the recognized one through glutamic-γ-semialdehyde. However N-acetylglutamic-γ-semialdehyde was isolated from the culture fluid of a mutant and in addition Vogel (192) reported the presence of a transaminase in a mutant and in the wild type which, in the presence of glutamic acid, transaminated the aldehyde group of N-acetylglutamic-γ-semialdehyde to form N-α-acetylornithine. Evidence that glutamic-γ-semialdehyde was not an in-

termediate in the synthesis of ornithine in *E. coli* was also presented. The proposed pathway (192) in *E. coli* is therefore glutamic acid→N-acetylglutamic acid→N-acetylglutamic-γ-semialdehyde→N-α-acetylornithine→ornithine. In contrast, Fincham (195) has reported a transaminase in *Neurospora* which will transaminate glutamic-γ-semialdehyde, to form ornithine.

Roche & Lacombe (196) extracted an arginine desmidase from yeast which formed one mole of citrulline and one of ammonia from one of arginine. It was concluded that the presence of this enzyme in cells which contained no arginase implied that arginine is not metabolized in the TCA cycle. The conversion of citrulline to ornithine by *S. faecalis* extracts was found by Slade (197) to require AMP or ADP in addition to the previously reported inorganic phosphate, and Mg or Mn. The reaction was inhibited by fluoride but iodoacetate, arsenite, and 2,4-dinitrophenol were without effect. Similar observations were reported by Oginsky & Gehrig (198) who, in addition, found that per mole of citrulline decomposed 1 mole of inorganic phosphate was converted to high energy phosphate. However, no new phosphate compound could be detected. Using extracts of a strain of *Pseudomonas*, Slade, Doughty & Slamp (199) confirmed the formation of high energy phosphate during the citrulline→ornithine reaction. The presence of a similar reaction in *Streptococcus lactis* was reported by Korzenovsky & Werkman (200). An excellent review of this problem and of the utilization of amino acids in general has been compiled by Slade (201).

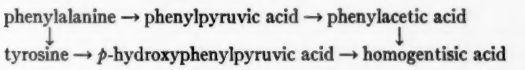
Additional evidence that histidine decarboxylase differs from other amino acid decarboxylases was obtained by Rodwell (202) who found that pyridoxal phosphate is apparently not necessary for the activity of the enzyme. Borek & Waelsch (203) found that extracts of *P. fluorescens* attacked α-formamidino glutaric acid, an intermediate formed by the action of liver homogenate on L-histidine or urocanic acid, more rapidly than histidine. On the basis of the distribution of labelled carbon in lysine isolated from cells of *T. utilis*, which had been grown on a glucose medium with additions of traces of methyl- and carboxyl-labelled acetate, Strassman & Weinhouse (204) postulated two pathways of lysine synthesis. In the first the methyl group of acetate may condense with the carbonyl carbon of an unsymmetrical succinate derivative such as succinyl-CoA. In the second the acetate methyl carbon may condense with the carbonyl carbon of α-ketoglutarate to give a seven-carbon homologue of citric acid, which by undergoing a sequence of reactions, analogous to those of the TCA cycle, could yield the lysine precursor, α-aminoadipic acid.

Weisendanger & Nisman (205) separated an enzyme fraction from extracts of *Clostridium sporogenes* which deaminated and simultaneously detached the methyl mercaptan group of methionine. The system was completely inactive in the absence of pyridoxal phosphate and was inactive on D-methionine. The enzymes differ from those found in *Neurospora* in that threonine but not serine is deaminated. Schlenk & Smith (206, 207) found that methionine acted as a specific precursor for the formation of thiomethyl adenosine in *T. utilis*. The labelled methyl or sulfur was found almost quan-

titatively in the nucleoside while the rest of the molecule was not transferred. The data are explained on the basis of the intermediate formation of S-adenosyl methionine which is split to thiomethyladenosine and homoserine.

Kearney & Singer (208, 209, 210) prepared a soluble enzyme extract from *Proteus vulgaris* which rapidly oxidized L-cysteinesulfinic acid. The metabolism of this compound was shown to proceed by two competing pathways. In the first pathway it was oxidized to cysteic acid which was then transaminated with α-ketoglutarate to yield β-sulfinylpyruvic acid. The second pathway was initiated by transamination of cysteinesulfinate. The resulting β-sulfinylpyruvic acid was desulfinated to pyruvate and $SO_3^=$. L-Cysteinesulfinic acid dehydrogenase required a new coenzyme for activity. The new coenzyme, provisionally called coenzyme III, was extensively purified from yeast. It would appear to be a pyridine nucleotide and transfers H^+ and electrons by virtue of the pyridine structure. This cofactor is tentatively suggested to be nicotinamide-ribose-(5)-pyrophosphate.

Making use of simultaneous adaptation and isolation techniques Dagley, Fewster & Happold (211) were able to show that a strain of *Vibrio* oxidized phenylalanine by two pathways.



They concluded that the pathway through phenylpyruvate is the major one since this compound could be isolated in quantity, unmixed with *p*-hydroxyphenylpyruvate.

DeBusk & Wagner (212) reported that, in a strain of *Neurospora*, many amino acids such as phenylalanine, inhibited the utilization of tyrosine. Since the inhibitory powers were common to so many compounds, it was suggested that interference with some general reaction, such as transamination, might be occurring. In confirmation of this was the finding that the deamination product of tyrosine, *p*-hydroxyphenylalanine, could reverse the inhibition caused by the amino acids. It appeared, therefore, that this compound has an essential function in the metabolism of the organism other than as a precursor of tyrosine. On the basis of experiments on the utilization of labelled acetate and pyruvate by yeast, Thomas *et al.* (213) postulated a mechanism for the formation of tyrosine. In confirmation of earlier work it was concluded that the aromatic ring was formed from pyruvate via oxaloacetate or similar unsymmetrical four-carbon acid. The side chain on the other hand appeared to arise from pyruvate as an intact unit. Miller, Tsuchida & Adelberg (214) found that extracts of a species of *Pseudomonas* contained a transaminase which converted L-kynurenine and α-ketoglutaric acid to kynurenic acid and glutamic acid. In the absence of α-ketoglutarate the extracts attacked kynurenine to form anthranilic acid. This observation corrects an earlier report (215) that the quinoline and aromatic pathways for the degradation of hynurenine were mutally exclusive.

Purines and pyrimidines.—In agreement with results now well estab-

lished with animal tissue and *Neurospora* Sutton & Werkman (216) found that in *A. aerogenes* glycine was the precursor of positions four, five, and seven of adenine and guanine. Also that CO_2 was incorporated into position six. Lieberman & Kornberg (217, 218) reported that cell-free extracts from an anaerobic soil bacterium carried out the following sequence of reactions.

$$\text{ortoic acid} + DPNH_2 \rightleftarrows \text{dihydroortoic acid} + DPN \qquad 22.$$

$$\text{dihydroortoic acid} + H_2O \rightleftarrows \text{ureidosuccinic acid} \qquad 23.$$

$$\text{ureidosuccinic acid} \rightarrow \text{5-acetic acid hydantoin} \qquad 24.$$

The enzyme for the first reaction, dihydroortoic dehydrogenase, was partially purified and the quantitative accumulation of dihydroortoic acid demonstrated.

In a study of the ribonucleic acids of *S. cerevisiae* Mallette & Lamanna (219) observed that, even with the best methods of isolation, a range of molecules of varying molecular weight were present. It was suggested that these differences in the structure of nucleic acids might indicate a degree of specificity in the combination of ribonucleic acid and protein during the synthesis of nucleoprotein.

LITERATURE CITED

1. Cohn, M., and Capdupuy, J., quoted by Cohn, M., and Monod, J., in *Adaptation in Microorganisms* (Gale, E. F., and Davis, J. G., Eds., Cambridge University Press, London, England, 340 pp., 1953)
2. Benzer, S., *Biochim. et Biophys. Acta*, **11**, 383–95 (1953)
3. Koppel, J. L., Porter, C. J., and Crocker, B. F., *J. Gen. Physiol.*, **36**, 703 (1953)
4. Porter, C. J., Holmes, R., and Crocker, B. F., *J. Gen. Physiol.*, **37**, 271–89 (1953)
5. Siminovitch, L., and Jacob, F., *Ann. inst. Pasteur*, **83**, 745–54 (1952)
6. Monod, J., and Wollman, E., *Ann. inst. Pasteur*, **73**, 937–56 (1947)
7. Jacob, F., *Ann. inst. Pasteur*, **82**, 578–702 (1952)
8. Cohn, M., and Torriani, A. M., *J. Immunol.*, **69**, 471–91 (1952)
9. Cohn, M., and Torriani, A. M., *Biochim. et Biophys. Acta*, **10**, 280–89 (1953)
10. Halvorson, H. O., and Spiegelman, S., *J. Bact.*, **65**, 496–504 (1953)
11. Halvorson, H. O., and Spiegelman, S., *J. Bact.*, **65**, 601–8 (1953)
12. Monod, J., Pappenheimer, A. M., Jr., and Cohen-Bazire, G., *Biochim. et Biophys. Acta*, **9**, 648–60 (1952)
13. Monod, J., Cohen-Bazire, G., and Cohn, M., *Biochim. et Biophys. Acta*, **7**, 585–99 (1951)
14. Pollock, M. R., *Brit. J. Exp. Pathol.*, **31**, 739–53 (1950)
15. Pollock, M. R., in *Adaptation in Microorganisms* (Gale, E. F., and Davis, J. G., Eds., Cambridge Uhiversity Press, London, England, 340 pp., 1953)
16. Cohn, M., and Monod, J., in *Adaptation in Microorganisms* (Gale, E. F., and Davis, J. G., Eds., Cambridge University Press, London, England, 340 pp., 1953)
17. Monod, J., and Cohen-Bazire, G., *Compt. rend.*, **236**, 530–32 (1953)
18. Monod, J., and Cohen-Bazire, G., *Compt. rend.*, **236**, 417–19 (1953)
19. Cohn, M., Cohen, G. N., and Monod, J., *Compt. rend.*, **236**, 746–48 (1053)
20. Bernheim, F., *J. Biol. Chem.*, **203**, 775–80 (1953)
21. Bernheim, F., and DeTurk, W. E., *J. Bact.*, **65**, 65–68 (1953)
22. Kaplan, N. O., Colowick, S. P., Neufeld, E. F., and Ciotti, M. M., *J. Biol. Chem.*, **205**, 17–29 (1953)
23. Kaplan, N. O., Colowick, S. P., Zatman, L. J., and Ciotti, M. M., *J. Biol. Chem.*, **205**, 31–43 (1953)
24. Fisher, H. F., Conn, E. C., Vennesland, B., and Westheimer, F. H., *J. Biol. Chem.*, **202**, 687–97 (1953)
25. Saz, A. K., and Slie, R. B., *J. Am. Chem. Soc.*, **75**, 4626–27 (1953)
26. Brodie, A. F., *J. Biol. Chem.*, **199**, 835–44 (1953)
27. Dolin, M. I., *Arch. Biochem. and Biophys.*, **46**, 483–85 (1953)
28. Krebs, E. G., *J. Biol. Chem.*, **200**, 471–78 (1953)
29. Krebs, E. G., Rafter, G. W., and Junge, J. M., *J. Biol. Chem.*, **200**, 479–92 (1953)
30. Velick, S. F., and Hayes, J. E., Jr., *J. Biol. Chem.*, **203**, 545–62 (1953)
31. Velick, S. F., and Udenfriend, S., *J. Biol. Chem.*, **203**, 575–82 (1953)
32. Velick, S. F., *J. Biol. Chem.*, **203**, 563–73 (1953)
33. Wood, W. A., and Schwerdt, R. F., *J. Biol. Chem.*, **201**, 501–11 (1953)
34. Entner, N., and Stanier, R. Y., *J. Bact.*, **62**, 181–86 (1951)
35. Stokes, F. N., and Campbell, J. J. R., *Arch. Biochem.*, **30**, 121–25 (1951)
36. Claridge, C. A., and Werkman, C. H., *Arch. Biochem. and Biophys.*, **47**, 99–106 (1953)

37. Lemoigne, M., Blachère, H., and Aubert, J.-P., *Compt. rend.*, **236**, 2188–91 (1953)
38. Aubert, J.-P., Milhaud, G., and Gavard, R., *Compt. rend.*, **235**, 1165–67 (1952)
39. Kitos, P. A., and Campbell, J. J. R. (Unpublished data)
40. Sokatch, J. T., and Gunsalus, I. C., *Abstracts Am. Chem. Soc., 124th Meeting* (Chicago, Ill., September, 1953)
41. Entner, N., and Doudoroff, M., *J. Biol. Chem.*, **196**, 853–62 (1952),
42. Wood, W. A., and Schwerdt, R. F., *J. Cellular Comp. Physiol.*, **41**, 165–82 (1953)
43. Katznelson, H., Tannenbaum, S. W., and Tatum, E. L., *J. Biol. Chem.*, **204**, 43–59 (1953)
44. Foda, O., and Vaughn, R. E., *J. Bact.*, **65**, 233–37 (1953)
45. Klein, H. P., *J. Bact.*, **66**, 120–21 (1953)
46. Scott, D. B. M., and Cohen, S. S., *Biochem. J.* (*London*), **55**, 23–33 (1953)
47. Scott, D. B. M., and Cohen, S. S., *Biochem. J.* (*London*), **55**, 33–36 (1953)
48. Sher, I. H., and Mallette, M. F., *J. Biol. Chem.*, **200**, 257–62 (1953)
49. Cochrane, V. W., Peck, H. D., Jr., and Harrison A., *J. Bact.*, **66**, 17–23 (1953)
50. Heath, E. C., and Koffler, H., *Abstracts Am. Chem. Soc., 124th Meeting* (Chicago, Ill., September, 1953)
51. DeFiebre, C. W., and Knight, S. G., *J. Bact.*, **66**, 170–72 (1953)
52. Blumenthal, H. J., Lewis, K. F., and Weinhouse, S., *Abstracts Am. Chem. Soc., 124th Meeting* (Chicago, Ill., September, 1953)
53. DeMoss, R. D., Gunsalus, I. C., and Bard, R. C., *J. Bact.*, **66**, 10–16 (1953)
54. Arnstein, H. R. V., and Bentley, R., *Biochem. J.* (*London*), **54**, 493–508 (1953)
55. Arnstein, H. R. V., and Bentley, R., *Biochem. J.*, (*London*) **54**, 508–16 (1953)
56. Arnstein, H. R. V., and Bentley, R., *Biochem, J.* (*London*), **54**, 517–22 (1953)
57. Krebs, H. A., Gurin, S., and Eggleston, L. V., *Biochem. J.* (*London*), **51**, 614–28 (1952)
58. Roberts, R. B., Cowie, D. B., Britten, R., Bolton, E., and Abelson, P. H., *Proc. Natl. Acad. Sci. U. S.*, **39**, 1013–19 (1953)
59. Eisenberg, M. A., *J. Biol. Chem.*, **203**, 815–36 (1953)
60. Delwiche, E. A., and Carson, S. F., *J. Bact.*, **65**, 318–21 (1953)
61. Cochrane, V. W., and Peck, H. D., Jr., *J. Bact.*, **65**, 37–44 (1953)
62. Barrett, J. T., and Kallio, R. E., *J. Bact.*, **66**, 517–25 (1953)
63. Mickelson, M. N., and Schuler, M. N., *J. Bact.*, **65**, 297–304 (1953)
64. Barban, S., and Ajl, S., *J. Bact.*, **66**, 68–71 (1953)
65. Dagley, S., and Dawes, E. A., *Nature*, **172**, 345–46 (1953)
66. Dagley, S., and Dawes, E. A., *J. Bact.*, **66**, 259–65 (1953)
67. Grunberg-Manago, M., and Gunsalus, I. C., *Bact. Proc.*, 73 (1953)
68. Englesberg, E., Gibor, A., and Levy, J. B., *Bact. Proc.*, 74 (1953)
69. Gillespie, D. G., and Gunsalus, I. C., *Bact. Proc.*, 80 (1953)
70. Foulkes, E. C., *Federation Proc.*, **12**, 203 (1953)
71. Foulkes, E. C., *Biochem. J.* (*London*), **54**, 323–28 (1953)
72. Campbell, J. J. R., Smith R. A., and Eagles, B. A., *Biochim. et Biophys. Acta*, **11**, 594 (1953)
73. Umbreit, W. W., *J. Bact.*, **66**, 74–81 (1953)
74. Rapoport, S., and Wagner, R. H., *Nature*, **168**, 295–96 (1951)
75. Lindstrom, E. S., *J. Bact.*, **65**, 565–70 (1953)
76. Lenhoff, H. M., and Kaplan, N. O., *Nature*, **172**, 730–32 (1953)

77. Pinchot, G. B., *J. Biol. Chem.*, **205,** 65–74 (1953)
78. Hirsch, H. M., *Biochim. et Biophys. Acta*, **9,** 674–86 (1952)
79. Haskins, F. A., Tissieres, A., Mitchell, H. K., and Mitchell, M. B., *J. Biol. Chem.*, **200,** 819–26 (1953)
80. Ycas, M., and Starr, T. J., *J. Bact.*, **65,** 83–88 (1953)
81. Keilin, D., *Nature*, **172,** 390–93 (1953)
82. Keilin, D., and Tissieres, A., *Nature*, **172,** 393–94 (1953)
83. Horecker, B. L., Smyrniotis, P. Z., and Klenow, H., *Federation Proc.*, **12,** 219 (1953)
84. Horecker, B. L., and Smyrniotis, P. Z., *J. Am. Chem. Soc.*, **75,** 2021–22 (1953)
85. Hochester, R. M., and Watson, R. W., *J. Am. Chem. Soc.*, **75,** 3284 (1953)
86. Mitsuhashi, S., and Lampen, J. O., *J. Biol. Chem.*, **204,** 1011–18 (1953)
87. Lampen, J. O., *J. Biol. Chem.*, **204,** 999–1010 (1953)
88. Cohen, S. S., *J. Biol. Chem.*, **201,** 71–84 (1953)
89. De la Haba, G., Leder, I. G., and Racker, E., *J. Am. Chem. Soc.*, **75,** 1010–11 (1953)
90. Lamanna, C., and Campbell, J. J. R., *J. Bact.*, **65,** 596–600 (1953)
91. Posternak, T., and Reymond, D., *Helv. Chim. Acta*, **36,** 260–68 (1953)
92. Anderson, L., Tamita, K., Kussi, P., and Kirkwood, S., *J. Biol. Chem.*, **204,** 769–80 (1953)
93. Hunter, G. J. E., *Biochem. J. (London)*, **55,** 320–28 (1953)
94. Asnis, R. E., and Brodie, A. F., *J. Biol. Chem.*, **203,** 153–59 (1953)
95. Magasanik, B., Brooke, M. S., and Karibian, D., *J. Bact.*, **66,** 611–19 (1953)
96. Neish, A. C., *Can. J. Botany*, **31,** 265–76 (1953)
97. Gibbs, M., and Gastel, R., *Arch. Biochem. and Biophys.*, **43,** 33–38 (1953)
98. Rutter, W. J., and Hansen, R. G., *J. Biol. Chem.*, **202,** 311–21 (1953)
99. Rutter, W. J., and Hansen, R. G., *J. Biol. Chem.*, **202,** 323–30 (1953)
100. Cabib, E., Leloir, L. F., and Cardini, C. E., *J. Biol. Chem.*, **203,** 1055–70 (1953)
101. Leloir, L. F., and Cabib, E., *J. Am. Chem. Soc.*, **75,** 5445–46, (1953)
102. Cardini, C. E., and Leloir, L. F., *Arch. Biochem. and Biophys.*, **45,** 55–64 (1953)
103. Bealing, F. J., and Bacon, J. S. D., *Biochem. J. (London)*, **53,** 277–85 (1953)
104. Pan, S. C., Nicholson, L. W., and Kolachov, P., *Arch. Biochem. and Biophys.*, **42,** 406–20 (1953)
105. Pan, S. C., Nicholson, L. W., and Kolachov, P., *Arch. Biochem. and Biophys.*, **42,** 421–34 (1953)
106. Jermyn, M. A., and Thomas, R., *Australian J. Biol. Sci.* **6,** 70–76 (1953)
107. Bealing, F. J., *Biochem. J. (London)*, **55,** 93–100 (1953)
108. Koepsell, H. J., Tsuchiya, H. M., Hellman, N. N., Kazenko, A., Hoffman, C. A., Sharp, E. S., and Jackson, R. W., *J. Biol. Chem.*, **200,** 793–801 (1953)
109. Hehre, E. J., *J. Am. Chem. Soc.*, **75,** 4866 (1953)
110. Gunsalus, I. C., *J. Cellular Comp. Physiol.*, **41,** 113–36 (1953)
111. Slater, E. C., *Ann. Rev. Biochem.*, **22,** 17–56 (1953)
112. Dolin, M., *Federation Proc.*, **12,** 198 (1953)
113. Strauss, B. S., *J. Am. Chem. Soc.*, **75,** 1012 (1953)
114. Seaman, G. R., *J. Bact.*, **65,** 744–45 (1953)
115. Seaman, G. R., *Proc. Soc. Exptl. Biol. Med.*, **82,** 184–89 (1953)
116. Reed, L. J., and DeBusk, B. G., *J. Am. Chem. Soc.*, **75,** 1261–62 (1953)
117. DeBusk, B. G., and Reed, L. J., *Federation Proc.*, **12,** 193–94 (1953)

118. Hager, L. P., and Gunsalus, I. C., *J. Am. Chem. Soc.*, **75,** 5767 (1953)
119. Reed, L. J., Gunsalus, I. C., Schnakenberg, G. H. F., Soper, Q. F., Boaz, H. E., Kern, S. F., and Parke, T. V., *J. Am. Chem. Soc.*, **75,** 1267–70 (1953)
120. Reed, L. J., DeBusk, B. G., Hornberger, C. S., Jr., and Gunsalus, I. C., *J. Am. Chem. Soc.*, **75,** 1271–73 (1953)
121. Hornberger, C. S., Jr., Heitmiller, R. F., Gunsalus, I. C., Schnakenberg, G. H. F., and Reed, L. J., *J. Am. Chem. Soc.*, **75,** 1273–77 (1953)
122. Seaman, G. R., *J. Biol. Chem.*, **200,** 813–18 (1953)
123. Hill, C. H., *J. Bact.*, **65,** 578–80 (1953)
124. Conway, E. J., and O'Malley, E., *Biochem. J.*, (*London*) **54,** 154–63 (1953)
125. Delwiche, E. A., Phares, E. F., and Carson, S. F., *Federation Proc.*, **12,** 194 (1953)
126. Phares, E. F., Carson, S. F., and Delwiche, E. A., *Abstracts Am. Chem. Soc., 124th Meeting*, 4C (Chicago, Ill., September, 1953)
127. Utter, M. F., *Phosphorus Metabolism*, **2,** 646 (McElroy, W. D., and Glass, B., Eds., Johns Hopkins Press, Baltimore, Md., 930 pp., 1952)
128. Whiteley, H. R., *J. Am. Chem. Soc.*, **75,** 1518–19 (1953)
129. Whiteley, H. R., *Proc. Natl. Acad. Sci. U. S.*, **39,** 772–79 (1953)
130. Whiteley, H. R., *Proc. Natl. Acad. Sci., U. S.* **39,** 779–85 (1953)
131. Wood, H. G., and Leaver, F. W., *Biochem. et Biophys. Acta*, **12,** 207–22 (1953)
132. Hayaishi, O., *J. Am. Chem. Soc.*, **75,** 4367 (1953)
133. Lichstein, H. C., and Boyd, R. B., *Arch. Biochem. and Biophys.*, **44,** 475–83 (1953)
134. Takamiya, A., *J. Biochem.* (*Japan*), **40,** 407–14 (1953)
135. Takamiya, A., *J. Biochem.* (*Japan*), **40,** 415–19 (1953)
136. Crewther, W. G., *Australian J. Biol. Sci.*, **6,** 190–97 (1953)
137. Jefferson, W. E., and Foster, J. W., *J. Bact.*, **65,** 587–92 (1953)
138. Gest, H., Swim, H. E., and Barkulis, S. S., *Federation Proc.*, **12,** 207 (1953)
139. Barkulis, S. S., and Gest, H., *Biochem. et Biophys. Acta*, **11,** 306–8 (1953)
140. Crewther, W. G., *Australian J. Biol. Sci.*, **6,** 205–21 (1953)
141. Stewart, R. C., and Sevag, M. G., *Federation Proc.*, **12,** 275 (1953)
142. Jones, M. E., Lipmann, F., Hilz, H., and Lynen, F., *J. Am. Chem. Soc.*, **75,** 3285–86 (1953)
143. Seegmiller, J. E., *J. Biol. Chem.*, **201,** 629–37 (1953)
144. Stadtman, E. R., *J. Biol. Chem.*, **203,** 501–12 (1953)
145. Gale, G. R., *J. Bact.*, **63,** 273–78 (1952)
146. Sistrom, W. R., and Stanier, R. Y., *J. Bact.*, **66,** 404–6 (1953)
147. Gunsalus, I. C., Gunsalus, C. F., and Stanier, R. Y., *J. Bact.*, **66,** 538–42 (1953)
148. Stanier, R. Y., Gunsalus, I. C., and Gunsalus, C. F., *J. Bact.*, **66,** 543–47 (1953)
149. Gunsalus, C. F., Stanier, R. Y., and Gunsalus, I. C., *J. Bact.*, **66,** 548–63 (1953)
150. Gunter, S. E., *J. Bact.*, **66,** 341–46, (1953)
151. Sakamoto, Y., Uchida, M., and Ichihara, K., *Med. J. Osaka Univ.*, **3,** 477–86 (1953)
152. Uchida, M., Sakamoto, Y., and Ichihara, K., *Med. J. Osaka Univ.*, **3,** 487–95 (1953)
153. Kakihara, Y., and Ichihara, K., *Med. J. Osaka Univ.*, **3,** 497–507 (1953)
154. Uchida, M., Taketomo, Y., Kakihara, Y., and Ichihara, K., *Med. J. Osaka Univ.*, **3,** 509–19 (1953)
155. Walker, N., and Wiltshire, G. H., *J. Gen. Microbiol.*, **8,** 273–76 (1953)
156. Davis, B. D., and Mingioli, E. S., *J. Bact.*, **66,** 129–36 (1953)

157. Salamon, I. I., and Davis, B. D., *J. Am. Chem. Soc.*, **75,** 5567–71 (1953)
158. Weiss, U., Davis, B. D., and Mingioli, E. S., *J. Am. Chem. Soc.*, **75,** 5572–76 (1953)
159. McElroy, W. D., Hastings, J. W., Sonnenfeld, V., and Coulombre, J., *Science*, **118,** 385 (1953)
160. Strehler, B. L., and Cormier, M. J., *Arch. Biochen. and Biophys.*, **47,** 16–33 (1953)
161. Cormier, M. J., and Strehler, B. L., *J. Am. Chem. Soc.*, **75,** 4864–65 (1953)
162. Taniguchi, S., Mitsui, H., Toyoda, J., Yamada, T., and Egami, F., *J. Biochem. (Japan)*, **40,** 175–85 (1953)
163. Marshall, R. O., Dishburger, H. J., MacVicar, R., and Hallmark, G. D., *J. Bact.*, **66,** 254–58 (1953)
164. Allan, M. B., and Najjar, V. A., *Federation Proc.*, **12,** 167 (1953)
165. Najjar, V. A., and Allan, M. B., *Federation Proc.*, **12,** 250 (1953)
166. Nicholas, D. J. D., Nason, A., and McElroy, W. D., *Nature*, **172,** 34 (1953)
167. Nason, A., and Evans, H. J., *J. Biol. Chem.*, **202,** 655–73 (1953)
168. Hofman, T., and Lees, H., *Biochem. J., (London)*, **54,** 579–83 (1953)
169. Newton, J. W., Wilson, P. W., and Burris, R. H., *J. Biol. Chem.*, **204,** 445–51 (1953)
170. Hamilton, P. B., Magee, W. E., and Mortenson, L. E., *Bact Proc.*, **82** (1953)
171. Green, M., and Wilson, P. W., *J. Bact.*, **65,** 511–17 (1953)
172. Hyndman, L. A., Burris, R. H., and Wilson, P. W., *J. Bact.*, **65,** 522–31 (1953)
173. Rudman, D., and Meister, A., *J. Biol. Chem.*, **200,** 591–604 (1953)
174. Altenbern, R. A., and Housewright, R. D., *J. Biol. Chem.*, **204,** 159–67 (1953)
175. Roberts, E., Ayengar, P., and Posner, I., *J. Biol. Chem.*, **203,** 195–204 (1953)
176. Robinson, J., and Katznelson, H., *Nature*, **172,** 627 (1953)
177. Mamelak, R., and Quastel, J. H., *Biochim, et Biophys, Acta*, **12,** 103–20 (1953)
178. Katz, J., Lieberman, I., and Barker, H. A., *J. Biol. Chem.*, **200,** 417–29 (1953)
179. Katz, J., Lieberman, I., and Barker, H. A., *J. Biol. Chem.*, **200,** 431–41 (1953)
180. Stadtman, E. R., and White, F. H., Jr., *J. Am. Chem. Soc.*, **75,** 2022 (1953)
181. Maas, W. K., and Novelli, G. D., *Arch. Biochem. and Biophys.*, **43,** 236–38 (1953)
182. Snoke, J. E., *J. Am. Chem. Soc.*, **75,** 4872–73 (1953),
183. Samuels, P. J., *Biochem. J. (London)*, **55,** 441–44 (1953)
184. Lester, R. L., *J. Am. Chem. Soc.*, **75,** 5448–9 (1953)
185. Umbreit, W. W., and Heneage, P., *J. Biol. Chem.*, **201,** 15–20 (1953)
186. Yanofsky, C., and Reissig, J. L., *J. Biol. Chem.*, **202,** 567–77 (1953)
187. Delluva, A. M., *Arch. Biochem. and Biophys.*, **45,** 443–46 (1953)
188. Hirsch, M. L. and Cohen, G. N., *Compt. rend.*, **236,** 2338–40 (1953)
189. Black, S., and Gray, N. M., *J. Am. Chem. Soc.*, **75,** 2271–72 (1953)
190. Black, S., and Wright, N. G., *J. Am. Chem. Soc.*, **75,** 5766 (1953)
191. Abelson, P. H., Bolton, E., Britten, R., Cowie, D. B., and Roberts, R. B., *Proc. Natl. Acad. Sci. U. S.*, **39,** 1020–26 (1953)
192. Vogel, H. J., *Proc. Natl. Acad. Sci. U. S.*, **39,** 578–83 (1953)
193. Maas, W. K., Novelli, G. D., and Lipmann, F., *Proc. Natl. Acad. Sci. U. S.*, **39,** 1004–8 (1953)
194. Vogel, H. J., Abelson, P. H., and Bolton, E. T., *Biochem. et Biophys. Acta*, **11,** 584–85 (1953)
195. Fincham, J. R. S., *Biochem. J. (London)*, **53,** 313–20 (1953)
196. Roche, J., and Lacombe, G., *Biochem. et Biophys. Acta*, **9,** 687–92 (1952)
197. Slade, H. D., *Arch. Biochem. and Biophys.*, **42,** 204–11 (1953)

198. Oginsky, E. L., and Gehrig, R. F., *J. Biol. Chem.*, **204,** 712–29 (1953)
199. Slade, H. D., Doughty, C. C., and Slamp, W. C., *Arch. Biochem. and Biophys.*, **48,** 338–46 (1954)
200. Korzenovsky, M., and Werkman, C. H., *Arch. Biochem. and Biophys.*, **46,** 178–85 (1953)
201. Slade, H. D., *Streptococcal Infections* (Columbia University Press, New York, N. Y., 1954)
202. Rodwell, A. W., *J. Gen. Microbiol.*, **8,** 233–37 (1953)
203. Borek, B. A., and Waelsch, H., *J. Biol. Chem.*, **205,** 459–74 (1953)
204. Strassman, M., and Weinhouse, S., *J. Am. Chem. Soc.*, **75,** 1680–84 (1953)
205. Wiesendanger, S., and Nisman, B., *Compt. rend.*, **237,** 764–65 (1953)
206. Schlenk, F., and Smith, R. L., *Federation Proc.*, **12,** 265 (1953)
207. Schlenk, F., and Smith, R. L., *J. Biol. Chem.*, **204,** 27–34 (1953)
208. Kearney, E. B., and Singer, T. P., *Biochem, et Biophys. Acta*, **11,** 270–75 (1953)
209. Kearney, E. B., and Singer, T. P., *Biochem. et Biophys. Acta*, **11,** 276–89 (1953)
210. Singer, T. P., and Kearney, E. B., *Biochim, et Biophys. Acta*, **11,** 290–99 (1953)
211. Dagley, S., Fewster, M., and Happold, F. C., *J. Gen. Microbiol*, **8,** 1–7 (1953)
212. DeBusk, A. G., and Wagner, R. P., *J. Am. Chem. Soc.*, **75,** 5131 (1953)
213. Thomas, R. C., Cheldelin, V. H., Christensen, B. E., and Wang, C. H., *J. Am. Chem. Soc.*, **75,** 5554–56 (1953)
214. Miller, I. L., Tsuchida, M., and Adelberg, E. A., *J. Biol. Chem.*, **203,** 205–11 (1953)
215. Stanier, R. Y., and Hayaishi, O., *Science*, **114,** 326–30 (1951)
216. Sutton, W. B., and Werkman, C. H., *Arch. Biochem. and Biophys.*, **47,** 1–7 (1953)
217. Lieberman, I., and Kornberg, A., *Federation Proc.*, **12,** 239 (1953)
218. Lieberman, I., and Kornberg, A., *Biochem. et Biophys. Acta*, **12,** 223–34 (1953)
219. Mallette, M. F., and Lamanna, C., *Arch. Biochem. and Biophys.*, **47,** 174–89 (1953)

THE CHEMOAUTOTROPHIC AND PHOTOSYNTHETIC BACTERIA[1]

By C. B. van Niel

Hopkins Marine Station of Stanford University, Pacific Grove, California

Although the autotrophic bacteria present some fundamental problems in comparative biochemistry, the chemoautotrophs are still somewhat neglected, probably because they are believed to be difficult to handle. Recent improvements in culture methods should therefore make the organisms more accessible.

The outburst of activity initiated by Vogler, Umbreit, and co-workers some ten years ago (1, 2) soon came to a halt, and only during the last years have similar studies on the thiobacilli been resumed. These have emphasized the need for further work. A large number of papers have appeared on nitrifying and sulfur-oxidizing bacteria, many of them dealing with the activities of the organisms under natural conditions. The iron- and hydrogen bacteria have also been the subject of several publications. But on the whole our understanding of the large and varied group of "inorgoxidants" has not been greatly advanced.

In contrast, the photosynthetic bacteria have been studied intensively, especially the nonsulfur purple bacteria. Part of the contributions have led to a better integration of previous knowledge; besides, new properties have been discovered and are under active investigation in a number of places.

The Chemoautotrophic Bacteria

Under this heading will be discussed the recent work on the colorless sulfur bacteria, iron bacteria, nitrifying bacteria, and hydrogen and carbon monoxide oxidizing bacteria. Theoretically one might include the arsenite oxidizing bacteria; however, the studies of Turner (3) and Quastel & Scholefield (4) have not shown that these organisms can grow in the absence of organic substances, so it is not yet possible to consider them as (potential) autotrophs. Mention should be made also of the bacteria that can live at the expense of the oxidation of methane (5), formate (6, 7, 8), and oxalate (9, 10), since they might represent microorganisms that use organic substrates exclusively as a source of energy, while synthesizing their cell material entirely from CO_2. The existence of this mode of life was envisaged almost 40 years ago by Lebedev (11), and in their discusssion of the properties of *Vibrio oxaliticus* Bhat & Barker likewise concluded:

> Oxalate and formate are very simple substrates. The removal of two electrons from either compound will result in its conversion to carbon dioxide. This leads to

[1] The survey of literature pertaining to this review was completed in January, 1954.

the idea that the metabolism of *V. oxaliticus* may be similar to that of the autotrophic bacteria in the sense that energy is obtained by the oxidation of a simple compound, whereas the cell materials are built up from carbon dioxide. There is no a priori reason why the energy for the reduction of carbon dioxide could not be obtained from the oxidation of oxalate just as well as from the oxidation of hydrogen or nitrite, for example. There is no definite evidence at present, however, in favor of this view (9, p. 367).

This verdict is still applicable, even though experiments with $C^{14}O_2$ have been conducted on the most conspicuous formate oxidizing bacterium, *Hyphomicrobium vulgare*, whose remarkable morphology has been described in some detail by Engel & Mevius (12). When grown in a mineral medium with methanol and $C^{14}O_2$, the cells contain appreciable amounts of the carbon isotope (8), but the published data do not permit a computation of the relative incorporation into cell constituents of carbon from methanol and from CO_2. The amino acids obtained by hydrolysis of the cells followed by paper-chromatographic separation gave, it is true, comparable counts for C^{14} content of the individual components; but in the absence of determinations of specific activities this result cannot be interpreted to indicate a uniform distribution of C^{14} which, if observed, would constitute a strong argument in favor of a total synthesis from CO_2. Curious is the reported O_2-uptake by cell suspensions supplied with sodium formate (*ca.* 750 μl. for 36.8 μM; Fig. 14), which is nearly twice the amount required for complete substrate oxidation (412 μl.). Because the measurements were made without KOH in the manometer flasks (presence of KOH prevents oxidation!) it is probable that the increased alkalinity of the suspensions resulting from formate oxidation caused a considerable CO_2 retention. Failure to take this into account may have led to the use of an inappropriate vessel constant for calculating the O_2 consumption.

Distribution and taxonomy.—The ubiquity of the sulfur bacteria is again demonstrated by the studies of H. Winogradsky (13, 14, 15) with mud cultures prepared according to S. Winogradsky (16). Special associations of *Beggiatoa* and protozoa have been analyzed by Fauré-Fremiet (17).

On the basis of size distribution of the organisms in natural collections, Klas (18) has proposed a third species of *Thiophysa*, *T. gigantea*, to take its place beside *T. volutans* and *T. macrophysa*. A new genus, *Thiogloea*, has been created by Devidé (19) for certain oval to elongate colorless sulfur bacteria; in addition to the two new species, *T. ruttneri* and *T. ragusina*, it now accommodates Molisch' *Bacterium bovista* as *T. bovista*. Morphologically *Thiogloea* closely resembles the purple sulfur bacteria of the *Thiothece-Amoebobacter* group. But the statements that light does not influence the development of *Thiogloea* cultures, and that even dense masses appear entirely colorless, seem to justify Devidé's conclusions. Since the organisms have not yet been studied in pure culture, it has yet to be established, however, that they are autotrophs.

Thiobacillus ferrooxidans (20, 21) resembles *T. thiooxidans*, but presum-

ably lacks the ability to oxidize elementary sulfur, and can oxidize ferrous iron under conditions precluding autooxidation (low pH). Hence it may also be considered as an iron bacterium, and it has been established that the oxidation of ferrous iron is used as a source of energy for growth.

The first part of a new survey of the iron bacteria has appeared (22); meanwhile Razumov (23) has proposed a new *Gallionella* species, *G. klasmiensis*.

In addition to the classical *Nitrosomonas europaea* Bömeke (24) recognizes *N. oligocarbogenes*, distinguished by its somewhat larger size, greater sensitivity to high temperatures and sodium chloride, and lower CO_2 assimilation per unit amount of NH_3 oxidized.

Schatz & Bovell (25) have described a new, strictly aerobic hydrogen bacterium, *Hydrogenomonas facilis*, which appears potentially very useful. Denitrification with molecular hydrogen has been established for Beijerinck's *Micrococcus denitrificans* (26); anaerobic growth is obtained only when nitrate is supplied, although not yet in strictly inorganic media. Hydrogen oxidation coupled with sulfate reduction can be accomplished by a majority of strains of the obligatory anaerobic *Desulfovibrio desulfuricans*, and with some of these growth in completely mineral media has been ascertained (27 to 32). *Methanobacterium formicicum* and *Methanosarcina barkerii*, isolated and described by Schnellen (33), share with *Methanobacterium omelianskii* (33, 34) the ability to oxidize hydrogen with the simultaneous reduction of CO_2 to CH_4. The two first-named methane producers can use CO in place of H_2 (23, 35); and the aerobic pseudomonad, isolated by Kistner (36) from elective cultures with CO as the only oxidizable substrate, has in turn been found to oxidize H_2. Strictly autotrophic growth of this aerobe has not yet been reported; the media on which pure cultures were obtained and perpetuated still contained 0.02 per cent peptone.

Culture methods.—For studies of chemoautotrophs silica gels offer many advantages over agar media, but their preparation has generally been considered to be more cumbersome. Recently devised improvements, such as the use of ion-exchange resins instead of dialysis (37), and the removal of dissolved gases by boiling prior to gelation, a procedure which seems to permit subsequent autoclaving without impairment of the gel structure (38), may dispose of many previously encountered objections.

Isolation of filamentous sulfur and iron bacteria with the aid of dilute organic media, advocated more than a decade ago by Cataldi (39, 40), has yielded satisfactory results in the hands of Pringsheim (41, 42) and Stokes (43, 44); at present it appears to be the method of choice for these organisms. Special media have been devised for the cultivation of *Thiobacillus ferrooxidans* (20, 21, 45); they are characterized by a low initial pH which stabilizes the divalent iron.

A notable contribution has been the development of satisfactory media for *T. denitrificans*. Although crude cultures, in which denitrification proceeds with the simultaneous oxidation of sulfur or thiosulfate, are easily estab-

lished, the isolation in pure culture of chemoautotrophic, denitrifying thiobacilli has generally met with little success. Sijderius, who made a concerted effort in this direction, could isolate none but heterotrophic bacteria from his elective cultures (46). From personal experience the reviewer is inclined to believe that this outcome is by no means exceptional. This has raised the problem of accounting for the relative abundance of heterotrophs in media presumably suitable only for the denitrifying sulfur bacteria. Sijderius' conclusion that the former develop at the expense of organic matter synthesized by the autotropic thiobacilli in the culture seems unsatisfactory because this should imply that the assimilatory activity of the latter results in the excretion of organic matter rather than in the formation of new cells. Now the Baalsruds (47) have shown that *T. denitrificans* cannot grow with nitrate as the sole source of nitrogen but requires reduced nitrogen compounds, such as ammonium salts, for protein synthesis. It is, therefore, more reasonable to ascribe the earlier mentioned discrepancy in relative numbers of heterotrophic and autotrophic bacteria in the crude cultures to the slow production by the former of ammonia from nitrate which thus determines the development of the latter. By adding an ammonium salt to the culture media the dependency of the thiobacilli on the activities of heterotrophic organisms can be eliminated, and plates streaked from young and actively denitrifying liquid cultures show a preponderance of colonies of *T. denitrificans*. It may be mentioned in passing that the requirement for reduced nitrogen compounds may be characteristic also for other denitrifying bacteria; perhaps *M. denitrificans* is a case in point. For satisfactory cultures of *T. denitrificans* it is, furthermore, important to guard against the accumulation of even small amounts of nitrite which is surprisingly toxic for this organism, concentrations as low as 10^{-4} *M* causing marked inhibition. The best way to prevent nitrite accumulation appears to be the use of thiosulfate in a concentration at least 2.5 times that of the nitrate.

Sartory *et al.* (48, 49) have succeeded in isolating the stalked iron bacterium *Gallionella* by the use of strictly inorganic media with iron filings, thus corroborating Lieske's (50) and Teichmann's (51) earlier findings. It is hoped that this interesting group of organisms will soon be studied in more detail.

The isolation in pure culture of *Nitrosomonas* remains an accomplishment worthy of publication (52, 53). Initial building up of cultures by consecutive additions of ammonia seems a sound procedure; the growth rate of *Nitrosomonas*, whose generation time has been estimated at 31 hr. (54), 15½ hr. (55), and a minimum of from 5 to 6 hr. (56), is so low, and the total population attainable with a single dose of 0.1 per cent ammonium sulfate so small, that contaminating heterotrophs, often reaching densities of 10^7 cells per ml. even in purified "mineral media," otherwise cause serious trouble in the isolation. Since the nitrifying bacteria tend to cluster around $CaCO_3$ particles in the liquid media, Meiklejohn (57) has achieved a better dispersion of the cells by dissolving excess $CaCO_3$ in a current of CO_2 gas. Addition of iron to the media is indicated (58, 59); the need of nitrifying bacteria for other

metals is apparently so small that ordinarily the impurities in the ingredients used suffice to satisfy it; deliberate addition of mixtures of trace elements frequently causes inhibition (58).

Organic growth factors, though not strictly required, may accelerate early growth of *Nitrosomonas;* this could account for the favorable effect of "Nährstoff Heyden" (56). The identity of the active components has not been established; Lees suspects that biotin may be involved (60).

Yeast extract in amounts too small to be significant as oxidizable substrate markedly increases the growth of potential autotrophs, such as *Desulfovibrio* (61 to 66); apparently it is indispensable for anaerobic growth of *M. denitrificans* with H_2 as substrate. Wieringa's *Clostridium aceticum* (67), another anaerobic H_2-oxidizing bacterium, and possibly a mesophilic variant of *C. thermoaceticum* (68, 69), grows only when supplied with some B vitamins and glutamate (70).

Role of chemoautotrophs in nature. —As participants in the mineralization process the chemoautotrophs have long been considered as the ultimate agents for reconverting the mineral constituents of living organisms, often liberated in reduced form (NH_3, H_2S, H_2) through microbial decomposition processes, into fully oxidized compounds. Oxidation of NH_3 and H_2S occurs in stages with the temporary accumulation of partially oxidized products. In connection with the sulfur cycle this has recently taken on considerable importance as providing a possible means of increasing the supply of elementary sulfur. Following the discovery that sulfur can be harvested regularly from certain North African lakes, Butlin (73) (see also 71, 72) has recognized this possibility. An analysis of the situation indicates that H_2S is generated by sulfate reduction and subsequently oxidized by sulfur bacteria with intermediate deposition of sulfur. This has suggested the feasibility of increasing the extent of the former process by the addition of cheap oxidizable substrates, and of controlling the activity of the sulfur bacteria in order to augment the yield of sulfur. Also in the USSR this possibility is under investigation (74). The numerous studies on sulfate reduction provide a sound basis for further developments (28 to 32; 61 to 66; 75 to 82).

Bacterial sulfate reduction was long ago considered as a major factor in the formation of metal sulfide deposits (83); this view is supported by Miller's work (84). Anaerobic corrosion of iron is now attributed chiefly to the activities of H_2-oxidizing strains of *Desulfovibrio* (85, 86, 87).

Oxidation of metal sulfides can be accomplished by various sulfur bacteria, among which especially the acid-tolerant thiobacilli appear to play a prominent role, with *T. ferroxidans* causing in addition an oxidation of ferrous to ferric iron (88 to 95). The rate of oxidation of the sulfur compounds is greatly influenced by the degree of dispersion.

The soil perfusion technique, a valuable tool for studies on the dynamics of mixed microbial populations in their normal environment and first developed for investigating nitrification in soils (96), has also been applied to an analysis of thiosulfate oxidation (97). In soil samples perfused with a

thiosulfate solution a biological transformation (the process is suppressed by azide!) results in the formation of sulfur and sulfate, the rate increasing exponentially to the point where the soil is "saturated" with the causative organisms. The stepwise oxidation primarily yields either sulfate plus tetrathionate, or sulfate plus sulfur; the former is the more common, but the latter can be augmented by the use of high phosphate and thiosulfate concentrations.

The perfusion technique permits, in addition, the application of the principle of simultaneous adaptation (98). An instructive example is provided by the demonstration that soil perfused with a tetrathionate solution until a maximum rate of oxidation is established immediately causes oxidation of thiosulfate at a constant rate and without initial lag, indicating that the microflora responsible for tetrathionate oxidation is the same as that causing thiosulfate oxidation (97). Trithionate is similarly oxidized by such soils, but not dithionate. The conclusion that tetra- and trithionate are normal intermediate products in the bacterial thiosulfate oxidation is in full agreement with Vishniac's demonstration of the thiosulfate-tetrathionate-trithionate sequence under the influence of partially inactivated suspensions of *T. thioparus* (99, 100), and with the work of Parker & Prisk (101).

Soil nitrification has also been analyzed by perfusion studies (96; 102 to 106); an excellent review of this work has appeared (107). Ammonia oxidation takes place at soil surfaces where ammonium ions are adsorbed; this follows from the demonstration that the rate of nitrification is proportional to the fraction of ammonia adsorbed on base-exchange complexes and is independent of the ammonium ion concentration; and that neither the perfusate nor the soil solution exhibit appreciable nitrifying capacity . A soil sample saturated with respect to ammonia oxidation does not cause immediate nitrification at constant rate when perfused with amines, urea, or glycine. The time lag indicates that these substances are first decomposed by other organisms, a conclusion supported by studies on pure cultures of nitrifying bacteria. The same technique has been used to show that copper-enzyme poisons reduce the rate of nitrification, while the inhibition can be counteracted by perfusion with solutions of copper salts (108), and that organic substances in low concentration (0.1 per cent) do not retard the rate of nitrification under natural conditions (106).

Nitrification of urethanes has been studied by the same technique, coupled with manometric measurements of O_2 uptake by previously "saturated" soils (109). When perfusion with ethyl urethane has caused a constant rate of nitrate production the addition of substituted urethanes does not result in immediate resumption of nitrification, and such materials inhibit nitrate formation from ethyl urethane. The mechanism of the inhibition has not yet been established. The decomposition of ethyl urethane is a biological process since inoculation of a fresh soil sample with a small amount of previously enriched soil decreased the lag ordinarily observed. The manometric experiments indicate that ethyl urethane is first hydrolyzed to ethanol, am-

monia, and CO_2, followed by oxidation of ethanol to acetic acid and of ammonia to nitrate. Supporting evidence for this view is the fact that the rate of O_2 uptake, greater with ethanol than with urethane, is not increased by the simultaneous addition of the two substrates; acetaldehyde behaves in the same way. A preliminary decomposition of ethyl urethane to ethylamine and CO_2 is unlikely because the addition of ethylamine does not cause an increase in O_2 consumption. It is somewhat puzzling that the oxidation processes would stop at the stage of acetic acid, particularly because there are so many organisms in soil that can oxidize acetate; although the measured O_2 uptake with ethanol, acetaldehyde, and ethyl urethane seems consistent with the assumption of acetate as a final product, and acetate addition causes little if any increase in O_2 consumption, it will be necessary to isolate acetic acid from the urethane-treated soils before this conclusion can be accepted. It is possible that microorganisms causing acetate oxidation develop mainly in the perfusion solution, rather than attached to soil particles, and thus do not participate to any large extent in the oxidations carried out by the soil samples in manometric experiments. Microscopic examination of the perfusion solution, and plating on acetate media would go far to settle this point.

The new methods promise to add materially to our knowledge of microbial processes under natural conditions; they constitute a significant addition to the very limited number of techniques hitherto used in such studies.

Physiology and biochemistry of the chemoautotrophs.—Winogradsky's rigid definition of a chemoautotroph as an organism that does not require and cannot use organic substances for growth can at present be applied to only a few representatives: four species of *Thiobacillus* [*T. thioparus*, *T. thiooxidans*, *T. denitrificans*, and *T. ferroxidans* (21, 22, 47, 99, 100, 110)], and the nitrifying bacteria (111, 112). The iron bacterium, *Gallionella*, may perhaps be included (29, 48), but as long as natural mineral waters are used for preparing media to grow the organism, this cannot be considered as established. Besides, ultraviolet irraditaion of *T. thiooxidans* has yielded mutants that develop only in media supplied with yeast extract (113), and the favorable effect of complex organic materials on the growth of *Nitrosomonas* (56, 60) has been mentioned previously.

Furthermore, it seems that the inhibitory effect of organic substances on the nitrifying bacteria, often used to emphasize the peculiar nature of their metabolism, has been much overrated. Though an interpretation of results with impure cultures (106, 114, 115) is at best difficult, experiments with pure cultures (116) have shown that *Nitrosomonas* can tolerate organic acids, polyalcohols, and sugars in concentrations of from 0.3 to 0.6 *M;* amino acids have a more pronounced adverse effect. The strong inhibition of soil nitrification by methionine (107) has not been observed with washed suspensions of pure cultures of *Nitrosomonas* (117), but Jensen & Sörensen (118) have noted that growing cultures are inhibited by methionine concentrations as low as 2×10^{-4} *M*. The much greater toxicity of substances with SH-groups

(118) suggests that in experiments on soil nitrification other microorganisms may produce such compounds from methionine; the reverse, i.e. the disappearance of an early inhibition of soil nitrification by thiocyanate has been analyzed by Gleen (119), and ascribed to a microbial oxidation of thiocyanate to ammonium sulfate in which organisms similar to the thiobacilli may be involved.

While there is no evidence that any of the above-mentioned chemoautotrophs can use organic substrates in place of the specific inorganic ones for energy and growth, all other chemoautotrophic bacteria can do so. Leaving aside the filamentous iron bacteria, which still have to be shown to be at least potentially capable of autotrophic development and which in the absence of ferrous iron develop as typical *Sphaerotilus* (41, 42), all other "inorgoxidants" can be grown at the expense of organic substances. The exceptional behavior of the obligatory autotrophs still presents a fundamental problem.

Baalsrud & Baalsrud (110) have been unable to confirm the earlier reported separation in time of substrate oxidation and CO_2 assimilation by thiobacilli (1, 2). They used suspensions of *T. thioparus*, *T. thiooxidans*, and *T. denitrificans*, grown under optimal conditions and in apparently excellent state of physiological activity. A decrease in inorganic phosphate concentration of the medium was observed during thiosulfate oxidation by all three species; the changes were extremely small and occurred under conditions where simultaneous CO_2 assimilation proceeded as well as in CO_2-free media, though they were slightly more pronounced under the latter conditions. These results are in good agreement with those of Vogler & Umbreit (120), both qualitatively and quantitatively. But a manometrically detectable CO_2 assimilation was found only when the cells were simultaneously oxidizing thiosulfate or sulfur. The conservative conclusions of the Baalsruds are that

> at present there appears to be no direct and convincing experimental support for the mechanism of chemosynthetic CO_2 assimilation proposed by Vogler and Umbreit. Our experiments have shown that in suspensions of *Thiobacillus* species CO_2 assimilation can only be detected if the organisms are simultaneously carrying out an oxidation of their natural substrate, and thus indicate that a much more intimate connection exists between the oxidative and assimilatory reactions than has lately been assumed. (110, p. 575).

The enzymes concerned in the oxidation of sulfur compounds have not yet been isolated. There is, however, good evidence to show that thiosulfate is first oxidized to tetrathionate by a "thiosulfate dehydrogenase" (47, 99, 100, 101, 121) and that trithionate is a product of tetrathionate oxidation. With damaged cell suspensions of pure cultures it is possible to observe oxidation of thiosulfate only as far as tetrathionate (47, 99, 100); the same effect can be obtained by the addition of 10^{-3} *M* NaF in aerobic experiments (121). The production of sulfur in thiosulfate media must be ascribed, as Nathansohn (122) had argued as early as 1902, to a strictly chemical interaction between excess thiosulfate and tetrathionate (99, 100).

The mechanism of oxidation of elementary sulfur by the thiobacilli has not yet been satisfactorily resolved. Since Postgate found *Desulfovibrio* unable to reduce sulfur purified by redistillation (78), it is conceivable that impurities are required to initiate also the oxidation by *Thiobacillus*, yielding products which in turn can react with sulfur to form metabolizable polythionates.

Oxidation of sulfur by *T. denitrificans* is very sensitive to nitrite which is approximately as toxic as cyanide; but thiosulfate oxidation proceeds rapidly in the presence of much higher nitrite concentrations, and, surprisingly, gives rise to NO formation; from nitrite and tetrathionate NO is not produced (47). This phenomenon deserves further study. It is the first case of recorded NO liberation by pure cultures of denitrifying bacteria and may play a role in the evolution of NO_2, occasionally observed in molasses fermentations, which has been ascribed to nitrite production by nitrate-reducing bacteria, followed by a chemical decomposition of the nitrite in an acid medium (123). This is a somewhat doubtful interpretation in view of the relatively low concentrations of nitrite and acid encountered in such fermentations.

The oxidation of ammonia by *Nitrosomonas* is probably catalyzed by metal enzymes, as indicated by the inhibitory effects of chelating agents (124). It also seems likely that hydroxylamine is the first product of ammonia oxidation (125, 126), as postulated by Kluyver & Donker in 1926 (127). Ordinarily, NH_3 disappears from suspensions of *Nitrosomonas* at the same rate at which nitrite accumulates, and the measured O_2 consumption quantitatively agrees with an oxidation in which incomplete oxidation products do not appear. But in the presence of 3×10^{-3} *M* hydrazine, nitrite is not formed from ammonia; in this case production of hydroxylamine can be detected. Furthermore, *Nitrosomonas* can oxidize hydroxylamine provided it is supplied in low concentrations (less than 10^{-4} *M*), and allylthiourea, a potent inhibitor of ammonia oxidation, has but little effect on the oxidation of hydroxylamine to nitrite.

In earlier perfusion experiments it had been found that the simultaneous addition of pyruvate and hydroxylamine caused nitrate formation (103), leading to the discovery that soil microorganisms can produce nitrite from oximes of α-ketoacids. This is not a property of *Nitrosomonas*, however (128); later work has shown that several types of heterotrophic bacteria can convert oximes with the liberation of nitrite (129, 130). At present it seems unlikely that amino acids, ammonia, or hydroxylamine are intermediate products in these decompositions, and a primary oxidation of oximes to nitrite and α-ketoacid appears more probable (131).

Certain methane-oxidizing bacteria have been reported to form nitrite from ammonia (132). Since only *Nitrosomonas* has so far been unambiguously implicated in this oxidation, it will be interesting to test pure cultures of this organism for its ability to oxidize methane.

Studies on the hydrogen bacteria have shown that *Hydrogenomonas facilis*, in contrast to other species, produces hydrogenase as a constitutive

enzyme and retains its ability to grow autotropically after years of subculturing on organic media (25, 26, 133, 134). Nevertheless, cultivation on organic substrates in air seems to suppress hydrogenase formation (135), probably because of the high O_2 tension which inhibits hydrogenase even in resting cell suspensions (25, 133, 134, 136). This inhibition has been ascribed to the involvement of SH-groups; but Gest (137), studying the inactivation of soluble hydrogenase preparations from *Escherichia coli* by O_2, failed to achieve reactivation by incubation with cysteine and other reducing agents or inhibition by *p*-chloromercuribenzoate. In the case of *H. facilis*, hydrogenase activity impaired by brief exposure to high O_2 tensions can be restored by incubation under H_2 with 5 percent O_2 (25).

Autotrophically grown *H. flava* oxidizes H_2 and lactate simultaneously (134); this holds also for *H. facilis* (135, 138). Apart from the obvious inference that hydrogenase is not concerned in lactate oxidation, these results

TABLE I*

SIMULTANEOUS OXIDATION OF H_2 AND LACTATE BY *H. facilis*

H. facilis grown with	O_2 consumption in μl. per 240 min; substrate				O_2 used in moles per mole of lactate; substrate:	
	H_2	Lact.	H_2 plus Lact.			
			Calc.	Found	Lact.	H_2+Lact.
H_2	230	380	610	490	3.2	2.3
Peptone	216	304	520	350	4.9	2.0
H_2+lactate	213	206	420	590	3.4	6.0

* Data calculated from Figures 1, 2, and 3 (135); values not corrected for endogenous metabolism.

imply that the ultimate O_2-activating enzyme system has a capacity exceeding that required for the maximum rate of oxidation of either substrate. The data of Wilson *et al.* (135) on the simultaneous oxidation of H_2 and lactate indicate that the O_2 consumption is not always additive, and comparison of the computed O_2 utilization per mole of lactate in the absence and presence of H_2 suggests that the rate of H_2 oxidation may be either reduced or enhanced (Table I); the latter effect has been observed by Kluyver & Manten (134); the former may reflect a limiting capacity of the cytochrome system. Experiments with *H. facilis* in the presence of $C^{14}O_2$ have shown that during lactate oxidation far more CO_2 is assimilated than is commensurate with ordinary "heterotrophic fixation" (138).

H. facilis can oxidize H_2 anaerobically in the presence of methylene blue or nitrate; the latter is quantitatively reduced to nitrite (25, 133). But H_2 oxidation coupled to nitrate reduction does not permit CO_2 assimilation

(136); this explains failure of the organism to grow anaerobically in nitrate media, though nitrate is an adequate nitrogen source for aerobic growth (25).

Extraction and purification of hydrogenase from typical hydrogen bacteria, or from hydrogen oxidizing bacteria such as *Desulfovibrio*, *C. aceticum*, and methane producers, has not yet been reported; this precludes comparison with the enzyme from *E. coli* (137) or *Azotobacter* (139, 140). The considerable hydrogenase activity of *H. facilis* (Q_{H_2} per mg. dry weight up to 1,100, and 2,200, in the presence of methylene blue!) should make it a satisfactory source. Schlegel (133) has found H_2 oxidation with methylene blue insensitive to cyanide, as is the hydrogenase of *Lactobacillus delbrückii* (141), recently confirmed by Winfield (personal communication). Hydrogenase from *E. coli* and *Azotobacter* has been reported to be inhibited by cyanide (142, 143), but recent work (137, 144) has yielded contradictory results.

The remarkable property of *Methanosarcina barkerii* to grow autotrophically in an atmosphere of pure CO has been analyzed (33, 35). The organism carries out two kinds of reactions:

$$CO + H_2O \rightarrow H_2 + CO_2, \text{ and} \qquad 1.$$

$$CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O \qquad 2.$$

In the presence of KOH, when CO_2 is never present in more than trace amounts, reaction 1 occurs almost exclusively; reaction 2 has been observed in special experiments. Crude cultures exposed to pure CO occasionally yield CO_2 and acetic acid; this can be interpreted as the result of the combined activities of *M. barkerii* (reaction 1) and *C. aceticum* (reaction 3):

$$2\,CO_2 + 4\,H_2 \rightarrow CH_3COOH + 2\,H_2O, \qquad 3.$$

but the presence of *C. aceticum* in such cultures has not been verified (33).

At present the hydrogen bacteria stand out as the most "efficient" among chemoautotrophs. Nevertheless, the Baalsruds have shown that thiobacilli can assimilate more CO_2 per unit amount of sulfur compound oxidized than previously had been found with growing cultures; values up to 12 and to 5.5 moles, respectively, of CO_2 assimilated per 100 moles O_2 or 16 moles of nitrate used have been obtained (47, 110). The extent of CO_2 assimilation during denitrification with sulfur is proportionately greater than with thiosulfate (47). Also, in young cultures of *Nitrosomonas* a greater efficiency may obtain than the generally accepted 5 per cent (145).

A beginning has been made with the analysis of cells of chemoautotrophs. A number of amino acids and carbohydrates have been chromatographically identified in hydrolysates of *Nitrosomonas* (146, 147). Except for the apparent absence of diaminopimelic acid, a regular component of all gram negative and most gram positive eubacteria (148, 149, 150), no marked differences with other bacteria have been noted. Chargaff *et al.* (151, 152, 153) have reported that the desoxypentose nucleic acids of a hitherto undescribed, gram positive hydrogen bacterium (referred to as "Bac. Schatz," though appar-

ently not a sporeformer) belong to the infrequent "GC" type in which the amounts of guanine and cytosine considerably exceed those of adenine and thymine.

T. thiooxidans has been used for the preparation of uniformly labeled amino acids by cultivation in the presence of $C^{14}O_2$; over-all yield was about 15 per cent of C^{14} supplied (154). For this purpose *T. dentrificans*, which has a greater assimilatory efficiency and, besides, is easier to grow in a closed system (thus permitting complete recovery of unused $C^{14}O_2$) now seems preferable, and *H. facilis* looks even better.

Assimilation of $C^{14}O_2$ during 40 min. by a suspension of *T. denitrificans* resulted in an approximately equal incorporation of C^{14} into water-soluble and nonsoluble substances (47). None of the products have yet been identified, but the Baalsruds' remark that "an analysis of the assimilatory mechanism in *T. denitrificans* with the techniques developed by Calvin and collaborators for the study of the gradual transformation of CO_2 in photosynthesis is certainly feasible" (47, p. 60) is fully justified. Studies of this kind have not yet been reported.

The Photosynthetic Bacteria

About 25 years ago the green and purple sulfur bacteria were definitely characterized as photosynthetic organisms; the nonsulfur purple and brown bacteria (155) soon afterwards could be included. The evidence has been summarized and discussed in recent reviews (156, 157).

The nonsulfur purple bacteria have been studied most; while the green sulfur bacteria have remained rather inaccessible largely for want of satisfactory culture methods. Larsen's extensive monograph on this group (158) has remedied this situation, and the green bacteria should now become useful for various sorts of investigations. The discovery of photochemical H_2 production by the purple bacteria has led to the demonstration that all the known photosynthetic bacteria can also fix molecular N_2.

Though it was the phototactic behavior of the purple bacteria that led Engelmann to surmise their photosynthetic ability in 1883 (159), detailed studies of this aspect have only recently been made, and these in turn have contributed significantly to an understanding of the mechanism of energy transfer between pigments. In general, much has been accomplished.

Distribution, culture methods, taxonomy, and morphology.—Green sulfur bacteria which, in contrast to previously isolated strains, can oxidize thiosulfate in addition to sulfide have been isolated in pure culture and described under the name *Chlorobium thiosulfatophilum* (158, 160). Recognition of special metal requirements has been used to design strictly inorganic culture media in which both *C. thiosulfatophilum* and *C. limicola* can be maintained under optimal conditions, with yields up to 2.7 gm. bacteria (wet weight) per liter (158). In such cultures the coiled filamentous growth forms, formerly considered typical for *C. limicola*, have not been observed; this is in agreement with Bicknell (161, 162).

Pringsheim (163, 164) has shown that some organisms previously called green bacteria are blue-green algae, and proposed a new genus, *Microchloris*, for the smallest green bacteria known today (*M. nadsonii*). The green and purple sulfur bacteria and the nonsulfur purple bacteria are provisionally assembled in an order, Chlorobacteriales, with the families Chlorobacteriaceae, Thiorhodaceae, and Athiorhodaceae (164). Pragmatically the advantage is that all photosynthetic bacteria thus comprise a single major taxon, but the reviewer doubts the justification of phylogenetic implications. Pringsheim further asserts that the green pigment of the green bacteria is identical with the bacteriochlorophyll of the purple bacteria (164, 165), and he uses this as an argument in support of a close relationship between the groups. This contention must be definitely rejected (see below).

A sulfur purple bacterium with unusual characteristics has been described by Scardovi (166) as *Rhodopseudomonas vannielli*. The original characterization (ability to grow in strictly mineral media with sulfide, optimum pH 6.5, maximum temperature 25°C., inability to photosynthesize with simple alcohols, acids, sugars, or amino acids, though developing in yeast autolysate media at the expense of unknown constituents) is at variance with later reports (personal communication) in which growth in peptone-meat extract media only at pH 8 or above is mentioned. Scardovi has supplied cultures of the organism on several occasions, but so far I have not been able to obtain subcultures from them.

Giesberger (167) has distinguished four species of *Rhodospirillum*, *R. rubrum*, *R. fulvum*, *R. molischianum*, and *R. photometricum*, on the basis of morphology and pigmentation. *Rhodomicrobium*, a new genus of nonsulfur purple bacteria (168), is morphologically a counterpart of the colorless *Hyphomicrobium* and develops by budding instead of fission (169). It is occasionally found in elective cultures in sulfide media; ethanol is the preferred substrate. In contrast to all other known nonsulfur purple bacteria, it does not require organic growth factors. In this connection it may be mentioned that the growth factor requirements of this group are identical for aerobic and anaerobic development (170, 171).

Various sulfur purple bacteria encountered in crude cultures have been described (14), and media for growing *Chromatium minus*, *C. gracile*, and *C. weissei* devised (172).

The pigments of the photosynthetic bacteria occur in well-defined structures, "chromatophores" (173, 174), or "grana" (175); these elements can be obtained by grinding, explosion, or supersonic oscillations of the cells followed by differential centrifugation. Thomas (175) mentions variations in size of the grana, but the studies of Pardee *et al.* (173, 174) indicate remarkable uniformity. From the size of the flattened disks seen on electron micrographs (0.11 μ) and the sedimentation constant (200 S) a diameter of from 0.03 to 0.06 μ has been computed. Each cell of *R. rubrum* contains about 5,000 such particles in which both the chlorophyllous and carotenoid pigments are concentrated as protein conjugates. Cells of *R. rubrum* grown in

darkness, when pigment production is suppressed, do not contain grana (174).

Physiology and biochemistry.—A photosynthetic conversion of secondary alcohols to the corresponding ketones with the assimilation of CO_2 by nonsulfur purple bacteria (176, 177) has been confirmed in the case of isopropanol with a strain of *Rhodopseudomonas gelatinosa* (178). Acetone itself is subject to further oxidation (179), and from simultaneous adaptation experiments it has been inferred that this proceeds by way of acetoacetate. Acetone produced by cells grown with unlabeled isopropanol and $C^{14}O_2$ is unlabeled; uniformly C^{14}-labeled cells likewise yield unlabeled acetone. These facts support the contention that acetone is formed by direct dehydrogenation of the substrate. But, as Gest (157) has emphasized, this does not mean that all organic substrates function merely as H-donors for a photochemical CO_2 reduction; that this had long ago been recognized is clear from the statement:

> This formulation (*i.e.*, that in bacterial photosyntheses organic substances can serve as H-donors with CO_2 acting as acceptor) does not, of course, imply that during the breakdown of these organic compounds there may not be formed intermediate products which can serve immediately as raw material for some anabolic reactions. It also does not exclude the possibility that CO_2 is not the only H-acceptor (180, p. 140–41).

And, though accumulated evidence favors the concept that substrate oxidation by illuminated purple bacteria with concomitant CO_2 assimilation is catalyzed by the same enzymes that induce oxidation in darkness in the presence of O_2 (181), the studies of Kamen *et al.* (182, 183) with methyl- and carboxyl-labeled acetate have shown that the fate of this substrate in light and dark metabolism is not identical. It is reasonable to interpret these observations, as Gest has done, to mean that "the reducing power generated by the action of light may significantly alter the final disposal of carbon intermediates derived from the accessory donor" (157, p. 195).

Studies on the oxidation of fatty acids by purple bacteria by Tsukamoto (184, 185) have shown that the rate of O_2 utilization increases with the chain length up to heptanoic acid; margaric and stearic acids apparently were not attacked. Irregularities similar to those noted by Gaffron (186, 187) for CO_2 assimilation with fatty acids as substrates were observed; thus, the total O_2 consumption for equivalent amounts of *iso*-acids was approximately twice as great as for the *N*-isomers, and comparable for propionate and butyrate. These results are difficult to interpret, all the more so because it is claimed that the oxidation of fatty acids in the dark does not give rise to CO_2 production and that in photosynthesis with acetate 0.25 moles of CO_2 were taken up per mole of acetate used. Both results are so much at variance with those obtained elsewhere that their validity must be questioned.

Since purple bacteria readily oxidize acetate in darkness only when supplied with CO_2 or trace amounts of dicarboxylic acids, and oxidation of

methyl- and carboxyl-labeled acetate results in an identical distribution of the isotopic carbon between CO_2 and assimilation products, oxidation by way of the Krebs cycle seemed likely (181). But citrate, isocitrate, and ketoglutarate are not oxidized (experiments with *R. rubrum*), and Tsukamoto, who found malonate noninhibitory (184) and has identified succinate and fumarate as products of acetate oxidation (185), has postulated a direct C_2 to C_2 condensation to form succinate. Nevertheless, recent evidence favors the earlier interpretation. Firstly, Kamen *et al.* (182) have shown that labeled citrate, ketoglutarate, and succinate are formed from labeled acetate. Secondly, Eisenberg (188) has convincingly demonstrated the presence of all enzymes known to function in the Krebs cycle in *R. rubrum*, and, because dried cells and extracts of this organism can oxidize all the members of the cycle, has concluded that failure of the living cells to do so must be attributed to a lack of contact between substrate and enzyme, perhaps as a result of permeability barriers. Finally, Elsden & Ormerod (189) have observed a strong inhibition of acetate oxidation by fluoroacetate with, in light, accumulation of citrate. The latter does not occur to an appreciable extent in darkness, once more emphasizing the possibility of a different fate of the substrate under the two conditions.

The different extent of cycling during metabolism in light and darkness may well be related to the enhanced synthetic reactions in light. These lead to a rapid formation of lipids, even at 0°C., and, in the presence of assimilable nitrogen, to protein (183). A detailed study of the amino acids produced during photosynthetic growth of *R. rubrum* in the presence of differentially labeled CO_2 and acetate ($C^{12}O_2+C^{13}H_3C^{14}OOH$, and $C^{14}O_2+C^{12}H_3C^{12}OOH$, respectively) has revealed that the methyl-C of acetate does not appear as carboxyl-C in alanine, aspartic and glutamic acids, or in the evolved CO_2 (190, 191, 192). This ingenious approach has also permitted the demonstration that the carboxyl-C of alanine stems from CO_2, and the methyl- and α-C from the methyl- and carboxyl-C of acetate, respectively, strongly suggesting a primary carboxylation of acetate to pyruvate. The latter, in turn, could be further carboxylated to a dicarboxylic acid, precursor of aspartic acid, in which the carbon atoms are distributed in conformity with the analytical results. The isotopic composition of the isolated glutamic acid agrees with that resulting from a condensation of acetate and a dicarboxylic acid, followed by a loss of CO_2, and eliminates a mechanism involving a carboxylation of a four-carbon compound. Together with the earlier mentioned lipid formation these results indicate that in the photosynthetic metabolism acetate can be used directly as a two-carbon building block for numerous syntheses. Since these would probably proceed by way of acetyl-CoA, it is possible that the reducing power generated by the photochemical reaction may be responsible for guiding a large proportion of the substrate into this path. It will therefore be of great interest to expand the studies of Ehrensvärd *et al.* by using culture media with substrates other than acetate, especially propionate. The significance of this substance derives from Larsen's

demonstration (158, 193) that green sulfur bacteria perform an extensive photochemical synthesis of succinate from propionate and CO_2. Although pyruvate was also photochemically converted by these organisms, succinate was not formed in this case; chromatographic analysis of the products has shown the appearance of two unidentified acidic substances, and the absence of simple fatty acids, succinic, fumaric, lactic, glyceric, citric, isocitric, *cis*- and *trans*-aconitic, ketoglutaric and phosphoglyceric acids, as well as simple amino acids (158, p. 175).

For an understanding of the photosynthetic assimilation of CO_2 into cell material Larsen's results have an additional, albeit largely negative, importance. Growth of the green sulfur bacteria in media with propionate and pyruvate instead of the "normal" oxidizable compounds, sulfide or thiosulfate, is negligible. Hence the substances produced from the organic substrates cannot be principally involved in the assimilation process. The work of Calvin *et al.* (194) on green plant photosynthesis has indicated that here, too, members of the Krebs cycle, though regularly encountered, are not primary assimilation products, while phosphoglyceric acid has come to occupy a central position; this substance is also the first labeled product of purple bacteria photosynthesis in the presence of $C^{14}O_2$ (183). After brief illumination of *R. rubrum* supplied with $C^{14}H_3COOH$ a different labeled compound ("X") has been found (183); in view of the importance now attached to sedoheptulose and ribulose phosphates as intermediate products in photosynthesis, and their discovery in experiments with purple bacteria (194, 195) it is possible that "X" may be related to these substances. Further discussion of the detailed mechanism of photosynthetic CO_2 assimilation can be omitted; an excellent review of this subject has recently appeared (196).

Quantum yield determinations for green bacteria photosynthesis in the presence of thiosulfate, tetrathionate, and H_2, respectively, have shown that in all three cases from 8 to 10 quanta are absorbed for the assimilation of a CO_2 molecule (158, 197). These results, in good agreement with determinations by Wassink *et al.* for purple bacteria (198), imply that the energy derived from the oxidation of the substrates is not available for CO_2 assimilation. With thin cell suspensions (1.3 to 2.3 mg. wet weight/ml.) the relation between rate of photosynthesis and light intensity is a linear function; with dense suspensions it is expressed by a sigmoid curve similar to that previously noted with purple bacteria (198, 199), where it must be attributed to H_2 production at low intensities (200). The green bacteria, too, carry out an anaerobic dark metabolism, but produce H_2S, CO_2, and unidentified acid, rather than H_2, from endogenous reserves.

The anaerobic decomposition of pyruvate by *R. rubrum* in darkness seems to depend on the previous history of the organism (201). Cells grown anaerobically under illumination in a malate-glutamate medium produce propionic and acetic acids and CO_2; cells grown aerobically in darkness produce in addition higher fatty acids. Fumarate and malate are fermented slowly, lactate and succinate not at all. It now seems possible that *R. rubrum*

can grow anaerobically in darkness when supplied with pyruvate, but this has not yet been shown.

These studies were an outgrowth of the earlier discovery by Gest & Kamen (202, 203) that *R. rubrum* can effect a light-dependent production of H_2. At first believed to be restricted to conditions where dicarboxylic acids were available, and, in the case of succinate, requiring the simultaneous presence of glutamate (204), photochemical H_2 evolution was later observed also as an endogenous process, magnified when substrates are present (205), sometimes to depend on the presence of CO_2 and to be exhibited by other nonsulfur as well as sulfur purple bacteria (206, 207). Parenthetically, it should be noted that in 1935 Roelofsen (208) recorded an endogenous H_2 production by *Chromatium* in darkness.

The mechanism is not understood. A photochemical decomposition of formate was considered, but early rejected. Later it was found that H_2 evolving suspensions can immediately decompose formate to H_2 and CO_2 in darkness, and that formate accumulates in H_2 producing cultures (201); this has led to doubt. And the fact that *R. gelatinosa* continues to liberate H_2 in the presence of malate or succinate, whereas addition of these substrates to suspensions that have ceased their endogenous H_2 production does not cause a resumption of gas evolution (205), suggests that it may represent a photodecomposition of accumulated cellular reserves, maintained at a high concentration when regular photosynthesis can proceed. This would also explain the need for CO_2 in certain cases and the failure to obtain extra H_2 production by *Chromatium* in the presence of thiosulfate (207), since in these experiments KOH was present, thus preventing photosynthesis with thiosulfate.

In the presence of NH_3 or N_2 purple bacteria do not produce H_2. The recognition that N_2 is not inert soon led to the demonstration of N_2 fixation (209, 210), now known to be a property of all photosynthetic bacteria (211 to 216). It has been suggested that inhibition of H_2 evolution by NH_3 and N_2 could be explained by postulating an increased amino acid synthesis for which hydrogen, instead of being liberated, is used as a reductant. But the lack of a stoichiometric relation between the "H_2 deficit" and NH_3 utilization (206) shows that much remains to be done.

Another unexplained phenomenon is the inhibitory effect of glycine on growth of *R. vanniellii*, reported by Scardovi (217). From the reviewer's records it is evident that many nonsulfur bacteria are similarly affected.

Like *T. thiooxidans*, the purple bacteria have been used for the synthesis of C^{14}-labeled proteins and amino acids (218); the advantages are that growth can be secured in bottle cultures so that much $C^{14}O_2$ can be supplied in a small space and that a better conversion (up to 40 per cent) is obtained. The disadvantages are that an organic substrate is required, resulting in a dilution of the labeled CO_2, and that the amino acids are not uniformly labeled (190, 191, 192). The ideal organisms for this purpose are evidently the green and purple sulfur bacteria, and the improved culture methods now available (158) should eliminate the objection that they are inconvenient.

Pigments, phototaxis, and photosynthesis.—Various photosynthetic bacteria show characteristic differences in their absorption spectra, attributable to differences in both the green (chlorophyllous) and yellow and red (carotenoid) pigments. The chlorophylls are responsible for the infrared bands, and, in the purple sulfur and nonsulfur bacteria, also for the absorption at 590 mμ.

In the purple bacteria several chlorophyll types have been found, with infrared absorption maxima at *ca.* 890, 870, 850, and 800 mμ; some species, like *R. rubrum*, contain only one; others, like *Chromatium*, as many as three types (219 to 224). Since the alcohol-extracted chlorophyll of all purple bacteria so far studied has identical spectral properties, and also since the phaeophytins obtained from different species are indistinguishable (155), it has generally been assumed that the different types in the intact cells or in the grana represent a variety of protein-complexes of one and the same bacteriochlorophyll.

The green sulfur bacteria contain a green pigment different from bacteriochlorophyll. So far only one type has been observed in cells and grana, with a main infrared absorption band at about 740 mμ (158, 219, 220), and no indication of a specific absorption at 590 mμ. In alcoholic extracts containing the free chlorophyll ("bacterioviridin," or "Chlorobium chlorophyll") the infrared band is shifted to *ca.* 670 mμ, and the phaeophytin, with its major peak at 668 mμ (in CCl_4), is equally distinct from the purple bacteria phaeophytin (760 mμ) (158, 224). These data, recently corroborated in unpublished experiments with R. Airth, make it impossible to accept Pringsheim's assertion that the green pigments of the green and purple bacteria are identical (164, 165).

Significant for an understanding of the mechanism of bacteriochlorophyll formation is Lascelles' observation that *R. spheroides* forms large amounts of porphyrins, chiefly coproporphyrin, when incubated in light with salts, glycine, ketoglutarate, and fumarate (225). The porphyrins appear in the medium, and may be identical with the previously observed but unidentified water-soluble red substances often encountered in cultures of nonsulfur purple bacteria (155). In the presence of iron salts (5.10^{-6} *M* or more) porphyrins are not formed, but an increased synthesis of bacteriochlorophyll takes place (see also 158); in darkness neither chlorophyll nor porphyrin is produced. The importance of glycine indicates that the mechanism of porphyrin synthesis is similar to that in animals.

The carotenoids of the photosynthetic bacteria are still poorly known. The only one that has been fairly well characterized is spirilloxanthin or rhodoviolascin (226), which has been considered as the only major carotenoid formed by *R. rubrum* (226, 227). Diphenylamine inhibits spirilloxanthin formation (228), as it does carotenoid synthesis in general (229, 230); grown in its presence *R. rubrum* produces less oxidized pigments (228). In *Rhodomicrobium* spirilloxanthin has been found, accompanied by β-carotene and what appear to be rhodopin and rhodovibrin (231); *R. spheroides*, in the

presence of O_2, converts a yellow carotenoid into a red one (232), but neither pigment is sufficiently known to permit a chemical interpretation.

Interest in the function of carotenoids has been stimulated by recent studies on phototaxis of purple bacteria. Phototactic action spectra have been determined for two strains of *R. rubrum* by independent methods, and with roughly concordant results (233 to 236); in agreement with Engelmann's early work (159) it has been shown that the spectral regions of maximum effectiveness coincide with those in which either the green or the red pigments exhibit absorption maxima. Since chlorophyll-absorbed radiation is obviously used in photosynthesis, Manten postulated that the infrared phototactic response was the result of a sudden drop in photosynthetic rate. Extrapolation of this principle to phototaxis in light chiefly absorbed by carotenoids would then imply that these pigments can participate directly in photosynthesis. This is now supported by several lines of evidence. Manten found that the ratio of contrast sensitivity of the shock reaction in infrared and green light, and hence the ratio of effectiveness of the two radiations, is constant over a wide range of intensities (233, 234). Deviations at high intensities were explained by the reasonable assumption that photosynthesis had reached a maximum rate (light saturation). By actual measurements of both photosynthesis and phototaxis Thomas & Nijenhuis (239) and Clayton (237) substantiated this. Clayton's experiments are particularly convincing because he induced variations in light saturation for photosynthesis by using different substrates and substrate mixtures. Temperature, cyanide, and urethane, which affect the rate of photosynthesis, have comparable effects on phototaxis (239). Finally, photosynthesis caused by carotenoid-absorbed light was directly demonstrated by Thomas (240) in manometric measurements with cell suspensions, and by Clayton (236), who showed that in spectral regions where absorption is attributable to either the chlorophyll or the carotenoids the growth rates of *R. rubrum* are identical if the relative intensities are adjusted to phototactic equivalence.

In studies on the quantitative relations between stimulus and response, Clayton (238) has found that purple bacteria phototaxis exhibits the general characteristics of biological irritability (all-or-none response, refractory period, accommodation) and that these relations can be expressed in complete formal analogy with the theories of excitation developed by Hill and by Rashevsky.

In one respect the phototactic action spectra of Manten and Clayton disagree, however, and for an interpretation of the role of carotenoids this is significant. Manten observed a minimum at 550 mμ, where spirilloxanthin has an absorption maximum; he therefore concluded that this pigment is not involved in either photosynthesis or phototaxis and attributed the effect of green light to absorption by another carotenoid with an absorption maximum at *ca.* 530 mμ where a phototactic maximum had been ascertained. Because spirilloxanthin has been considered as the only major carotenoid formed by *R. rubrum* (226, 227) this result was surprising, even though the

interpretation was strengthened by Thomas' measurements of photosynthetic activity as a function of wavelength (240), indicating a minimum at 550, and a maximum at 530 mμ. Clayton, on the other hand, found a maximum for phototactic activity at 550 mμ but did not determine an action spectrum for photosynthesis; his conclusion was:

> If both spectra are to be taken seriously they must be reconciled by the assumption that the two strains of *R. rubrum* employed differed widely in the activities or in the relative concentrations of their pigments. Both strains were isolated by van Niel; their absorption spectra as shown in Fig. 4 indicate the presence of spirilloxanthin and bacteriochlorophyll in roughly the same relative amounts. The scarcer components of the carotenoid complex do not appear in these relatively crude absorption spectra, and their concentrations in the two strains of bacteria could conceivably differ by a large factor. Such a difference would cause exactly the variation in action spectra with which we are confronted. Had the strain used by Manten been more deficient in the scarcer carotenoids, the maxima in his action spectrum at 490 and 530 mμ might have been suppressed to a level far below the chlorophyll peak at 590 mμ; at the same time the less pronounced maxima due to spirilloxanthin might have emerged from the masking influence of the other peaks. Conversely, an increase in the concentration of the scarcer carotenoids in (my) strain might have caused the present action spectrum to resemble that obtained by Manten.
>
> The assumption, required in the foregoing interpretation, of low spirilloxanthin activity coupled with high activity on the part of much less preponderant carotenoids is not very satisfying. The possibility remains, of course, that either or both action spectra were vitiated by uncontrolled environmental influences . . . " (236, pp. 115-16).

It was Duysens' account of a change in the absorption spectrum of *R. rubrum* with age of the culture (223) which indicated the involvement of uncontrolled variables. The spirilloxanthin band at 550 mμ, strikingly displayed by "old" cultures, is absent in "young" ones which instead show a minor absorption maximum at 530 mμ. The change has been confirmed with both strains used in the phototaxis studies; it may occur within 24 hr. (unpublished experiments with R. Airth). It also appears probable that the different culture media used in Utrecht (1 per cent peptone) and by Clayton (1 per cent pyruvate) have contributed to differences in pigmentation, since pyruvate greatly stimulates carotenogenesis in other bacteria (242; ref. from 243). Furthermore, exploratory experiments (with L. R. Blinks, unpublished) indicate that *R. rubrum* can photosynthesize with light absorbed by spirilloxanthin. The contrary claim (240) may therefore have been inspired by experiments with bacteria lacking this pigment; the absence of spirilloxanthin in at least some of the cultures used by Thomas is attested to by recently published absorption spectra (241).

Whether the carotenoids of the green bacteria can participate in photosynthesis is still an open question. The action spectrum determined by Larsen (158) does not permit a conclusion because the relative contribution of the chlorobium chlorophyll and the carotenoids to the absorption in the 400 to 500 mμ range cannot be estimated from available data.

Various species of purple bacteria contain more than one bacteriochlorophyll type (223), and the participation of at least some carotenoids in light absorption for photosynthesis has been established. This raises the problem of whether the primary photochemical act in the photosynthetic mechanism can be accomplished by each of these substances or whether only one is thus involved. By demonstrating that only the bacteriochlorophyll with an infrared absorption maximum at 890 mμ ("B 890") fluoresces, and that this fluorescence is excited by quanta absorbed by the other chlorophylls and by carotenoids, Duysens (223, 244) provided evidence for the occurrence of energy transfer between the pigments. From the observed fluorescence intensity it was concluded that this transfer occurs with an efficiency of about 100 per cent among the chlorophylls, and of about 40 per cent from carotenoids to "B 890." It thus seems probable that only "B 890" functions directly in the conversion of radiant into chemical energy. The mechanism of energy transfer can best be interpreted as internal conversion, as proposed by Arnold & Oppenheimer (245), or inductive resonance (223, 224). The concentration of the pigments in the cells, especially if their occurrence exclusively in grana or chromatophores (173, 174, 175) is taken into account, is sufficient to allow for this mechanism.

Recent developments indicate that also the primary photochemical reaction in photosynthesis may soon be better understood. It is no longer disputed that in essence it represents a photodecomposition of H_2O under the influence of excited chlorophyll with the formation of an oxidized and a reduced entity from which the various dark reactions take off, and future work can concentrate on establishing the nature of the enzymes involved.

The reviewer believes that Schenck's theory of the primary reaction (246) cannot apply to photosynthesis in general because the noninvolvement of O_2 in the bacterial photosyntheses has not been considered. Also the imaginative approach of Calvin (247), which seems to implicate thioctic acid in the first step during green plant photosynthesis, must still be extended to the photosynthetic bacteria.

On the other hand, the discovery by Hill *et al.* (248, 249, 250) of a new cytochrome (*f*) in chloroplasts of green plants has given impetus to studies on cytochromes in purple and green bacteria. Vernon (251) reported high concentrations of cytochrome *c* in *R. rubrum*, but Elsden, in independent investigations, had concluded that significant differences exist between cytochrome *c* and the bacterial haemochrome which also occurs in *R. spheroides;* this has since been corroborated (252). The presence of yet another cytochrome in green sulfur bacteria has been announced by Kamen (personal communication).

It is too early to assert that these cytochromes play a role in the primary photochemical reaction. The established photooxidation of cytochrome *c* by extracts of *R. rubrum* (253) is, at best, indicative. But Elsden (254) has pointed out that the properties of the new purple bacteria cytochrome are compatible with the requirements of a catalyst which, in the primary photo-

chemical reaction, becomes the "oxidized entity" and thus might be responsible for the increase in redox potential observed when suspensions of purple bacteria are illuminated in the absence of substrate (208, 255, 256).

In this connection a recent report of Duysens (257) is of great significance. Earlier experiments (223) had revealed a reversible change in infrared absorption when suspensions of purple bacteria were illuminated. By extending his studies to include measurements in the visible region, Duysens has now found that infrared irradiation of *R. rubrum* produces a reversible change in the absorption spectrum which corresponds almost exactly to that produced by the conversion from the reduced to the oxidized form of cytochrome *c;* the the slight shift (*ca.* 7 mμ) towards longer wavelengths obviously suggests that the new cytochrome rather than *c* is involved. It may be confidently expected that a further pursuit of these studies on the photosynthetic bacteria will contribute once again to a greater comprehension of photosynthesis in general.

LITERATURE CITED

1. Umbreit, W. W., *Bact. Revs.*, **11**, 157–66 (1947)
2. Umbreit, W. W., in *Bacterial Physiology*, 566–75 (C. H. Werkman and P. W. Wilson Eds., Academic Press, Inc., New York, N. Y., 707 pp., 1951)
3. Turner, A. W., *Nature*, **164**, 76–77 (1949)
4. Quastel, J. H., and Scholefield, P. G., *Soil Sci.*, **75**, 279–85 (1953)
5. Hutton, W. E., and ZoBell, C. E., *J. Bact.*, **58**, 463–73 (1949)
6. Stadtman, T. C., and Barker, H. A., *J. Bact.*, **62**, 269–80 (1951)
7. Boltjes, T. Y. Kingma, *Arch. Mikrobiol.*, **7**, 188–205 (1936)
8. Mevius, W., *Arch. Mikrobiol.*, **19**, 1–29 (1953)
9. Bhat, J. V., and Barker, H. A., *J. Bact.*, **55**, 359–68 (1948)
10. Müller, H., *Arch. Mikrobiol.*, **15**, 137–48 (1950)
11. Lebedev, A. F., *Amer. Rev. Soviet Med.*, **5**, 15–27 (1948)
12. Engel, H., and Mevius, W., *Ber. deut. botan. Ges.*, **64**, 208–12 (1951)
13. Winogradsky, H., and Appert, J., *Ann. inst. Pasteur*, **78**, 365–81 (1950)
14. Winogradsky, H., *Ann. inst. Pasteur*, **81**, 441–51 (1951)
15. Winogradsky, H., *Ann. inst. Pasteur*, **84**, 577–87 (1953)
16. Winogradsky, S., *Microbiologie du Sol*, 24–41; 83–126 (Masson & Cie., Paris, France, 861 pp., 1949)
17. Fauré-Fremiet, E., *Hydrobiologia*, **3**, 65–71 (1951)
18. Klas, Z., *Bull. intern. acad. yougoslave*, **3**, 5–13 (1951)
19. Devidé, Z., *Schweiz. Z. Hydrobiol.*, **14**, 446–55 (1952)
20. Colmer, A. R., Temple, K. L., and Hinckle, M. E., *J. Bact.*, **59**, 317–28 (1950)
21. Temple, K. L., and Colmer, A. R., *J. Bact.*, **62**, 605–11 (1951)
22. Beneden, G. van, *Hydrobiologia*, **3**, 1–64 (1951)
23. Razumov, A. S., *Mikrobiologiya*, **18**, 442–46 (1949)
24. Bömeke, H., *Arch. Mikrobiol.*, **15**, 414–27 (1951)
25. Schatz, A., and Bovell, C., *J. Bact.*, **63**, 87–98 (1952)
26. Kluyver, A. J., in *Symposium on microbial metabolism; Rend. ist. super. sanità, Rome*, 71–91 (1953)
27. Butlin, K. R., and Adams, M. E., *Nature*, **160**, 154–55 (1947)
28. Butlin, K. R., Adams, M. E., and Thomas, M., *J. Gen. Microbiol.*, **3**, 46–59 (1949)

29. Adams, M. E., Butlin, K. R., Hollands, S. J., and Postgate, J. R., *Research (London)*, **4**, 245–46 (1951)
30. Sisler, F. D., and ZoBell, C. E., *J. Bact.*, **60**, 747–56 (1950)
31. Sisler, F. D., and ZoBell, C. E., *J. Bact.*, **62**, 117–27 (1951)
32. Senez, J., and Volcani, B. E., *Compt. rend.*, **232**, 1035–36 (1951)
33. Schnellen, C. G. T. P., *Onderzoekingen over de methaangisting* (Doctoral thesis, Univ. of Delft, Delft, Holland, 137 pp., 1947)
34. Barker, H. A., *Proc. Natl. Acad. Sci. U. S.*, **29**, 184–90 (1943)
35. Kluyver, A. J., and Schnellen, C. G. T. P., *Arch. Biochem.*, **14**, 57–70 (1947)
36. Kistner, A., *Koninkl. Ned. Akad. Wetenschap. Proc.*, [C] 443–50 (1953)
37. Taylor, C. B., *J. Gen. Microbiol.*, **4**, 235–37 (1950)
38. Augier, J., and Pochon, J., *Ann. inst. Pasteur*, **85**, 666–70 (1953)
39. Cataldi, M. S., *Rev. inst. bacteriol. dep. nacl. hig.* (Buenos Aires), **9**, 393–423 (1940)
40. Cataldi, M. S., *Estudio fisiológico y sistemático de algunas Chlamydobacteriales* (Doctoral thesis, Univ. of Buenos Aires, Argentina, 97 pp., 1939)
41. Pringsheim, E. G., *Trans. Roy. Soc. London*, [B]**233**, 453–82 (1949)
42. Pringsheim, E. G., *Biol. Revs. Cambridge Phil. Soc.*, **24**, 200–45 (1949)
43. Stokes, J. L., *Bact. Proc.*, **18** (1953)
44. Stokes, J. L. (Personal communication on isolation of Beggiatoa)
45. Leathen, W. W., McIntyre, L. D., and Braley, S. A., *Science*, **114**, 280–81 (1951)
46. Sijderius, R., *Heterotrophe bacterien, die thiosulfaat oxydeeren* (Doctoral thesis, Univ. of Amsterdam, Amsterdam, Holland, 146 pp., 1946)
47. Baalsrud, K., and Baalsrud, K. S., *Arch. Mikrobiol.*, **20**, 34–62 (1954)
48. Sartory, A., and Meyer, J., *Compt. rend.*, **225**, 541–42 (1947)
49. Sartory, A., Sartory, R., and Meyer, J., 273–76, *Microbiologie pratique* (Librairie Maloine, Paris, France, 825 pp., 1950)
50. Lieske, R., *Jahrb. wiss. Botan.*, **49**, 91–127 (1911)
51. Teichmann, E., *Vergleichende Untersuchungen über die Kultur und Morphologie einiger Eisenorganismen* (Doctoral thesis, Deutsche Univ., Prag, Czechoslovakia, 1935) (cited in Pringsheim, E. G., *Biol. Revs. Cambridge Phil. Soc.*, **24**, 1949)
52. Meiklejohn, J., *Nature*, **164**, 667 (1949)
53. Lees, H., *Nature*, **167**, 355–56 (1951)
54. Buswell, A. M., van Meter, I., and Gerke, J. R., *Sewage and Ind. Wastes*, **22**, 508–13 (1950)
55. Bömeke, H., *Arch. Mikrobiol.*, **14**, 63–98 (1948)
56. Boltjes, T. Y. Kingma, *Nature*, **169**, 1106–7 (1952)
57. Meiklejohn, J., *J. Gen. Microbiol.*, **4**, 185–91 (1950)
58. Lees, H., and Meiklejohn, J., *Nature* **161**, 398–99 (1948)
59. Meiklejohn, J., *J. Gen. Microbiol.*, **8**, 58–65 (1953)
60. Lees, H., *Nature*, **169**, 1107 (1952)
61. Senez, J., *Ann. inst. Pasteur*, **80**, 395–408 (1951)
62. Senez, J., *Ann. inst. Pasteur*, **84**, 595–604 (1953)
63. Baumann, A., and Denk, V., *Arch. Mikrobiol.*, **15**, 283–307 (1950)
64. Miller, L. P., *Contribs. Boyce Thompson Inst.*, **15**(9), 467–74 (1949)
65. Postgate, J. R., *J. Gen. Microbiol.*, **5**, 714–24 (1951)
66. Postgate, J. R., *J. Gen. Microbiol.*, **9**, 440–44 (1953)
67. Wieringa, K. T., *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **6**, 251–62 (1940)

68. Barker, H. A., *Proc. Natl. Acad. Sci. U. S.*, **30**, 88–90 (1944)
69. Barker, H. A., and Kamen, M. D., *Proc. Natl. Acad. Sci. U. S.*, **31**, 219–25 (1945)
70. Karlsson, J. L., Volcani, B. E., and Barker, H. A., *J. Bact.*, **56**, 781–82 (1948)
71. Pratt, D. D., *Chemistry Research* (London), H.M. Stationery Office, 75–92; espec. pp. 85–89 (1950)
72. Pratt, D. D., *Chemistry Research* (London), H.M. Stationery Office, 97–112; espec. pp. 110–112 (1951)
73. Butlin, K. R., *Research*, **6**, 184–91 (1953)
74. Murzaev, P. M., *Compt. rend. acad. sci. U.R.S.S.*, **72**, 343–45 (1950)
75. Miller, L. P., *Contribs. Boyce Thompson Inst.*, **15**(9), 437–65 (1949)
76. Miller, L. P., *Contribs. Boyce Thompson Inst.*, **16**(3), 73–88 (1950)
77. Postgate, J., *Nature*, **164**, 670–71 (1949)
78. Postgate, J. R., *J. Gen. Microbiol.*, **5**, 725–38 (1951)
79. Postgate, J., *Research*, **5**, 189–90 (1952)
80. Postgate, J. R., *J. Gen. Microbiol.*, **6**, 128–42 (1952)
81. Starkey, R. L., *J. Am. Water Works Assoc.*, **40**, 1291–98 (1948)
82. Grossman, J., and Postgate, J. R., *Proc. Soc. Appl. Bact.*, **16**, 1–9 (1953)
83. Nadson, G., *Microorganismi kak geologitscheskie dieiateli* (*Microorganisms as geological factors*) (In Russian; St. Petersburg, Russia, 1903); cited in Waksman, S. A., *Principles of Soil Microbiology*, 2nd ed., Williams and Wilkins Co., Baltimore, Md., 894 pp., 1932)
84. Miller, L. P., *Contribs. Boyce Thompson Inst.*, **16**(3), 85–89 (1950)
85. Wight, K. M., and Starkey, R. L., *J. Bact.*, **50**, 238 (1945)
86. Starkey, R. L., *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **12**, 193–203 (1947)
87. Spruit, C. J. P., and Wanklyn, J. N., *Nature*, **168**, 951–52 (1951)
88. Gleen, H., *Nature*, **166**, 871–72 (1950)
89. Gleen, H., *J. Gen. Microbiol.*, **5**, xv (1951)
90. Quispel, A., Harmsen, G. W., and Otzen, D., *Plant and Soil*, **4**, 43–55 (1952)
91. Temple, K. C., and Delchamps, E. W., *Appl. Microbiol.*, **1**, 255–58 (1953)
92. Rabotnova, I. L., Ulebekova, M. V., and Magnitskaya, L. V., *Mikrobiologiya*, **19**, 402–9 (1950)
93. Leathen, W. W., Braley, S. A., and McIntyre, L. D., *Appl. Microbiol.*, **1**, 61–64 (1953)
94. Leathen, W. W., Braley, S. A., and McIntyre, L. D., *Appl. Microbiol.*, **1**, 65–68 (1953)
95. Bryner, L. C., Beck, J. V., Wilson, D. G., King, D., and Davis, D., *Bact. Proc.*, 17 (1953)
96. Lees, H., and Quastel, J. H., *Biochem. J.* (*London*), **40**, 803–15 (1946)
97. Gleen, H., and Quastel, J. H., *Appl. Microbiol.*, **1**, 70–77 (1953)
98. Stanier, R. Y., *J. Bact.*, **54**, 339–48 (1947)
99. Vishniac, W., *On the metabolism of the chemolithoautotrophic bacterium, Thiobacillus thioparus Beijerinck.* (Doctoral thesis, Stanford University, Stanford, Calif., 220 pp., 1949)
100. Vishniac, W., *J. Bact.*, **64**, 363–73 (1952)
101. Parker, C. D., and Prisk, J., *J. Gen. Microbiol.*, **8**, 344–64 (1953)
102. Lees, H., and Quastel, J. H., *Biochem.* (*London*), **40**, 815–23 (1946)
103. Lees, H., and Quastel, J. H., *Biochem. J.* (*London*), **40**, 824–28 (1946)
104. Stevenson, I. L., and Chase, F. E., *Soil. Sci.*, **76**, 107–14 (1953)

105. Lees, H., *Biochem. J.* (*London*), **42,** 534–38 (1948)
106. Lees, H., *Biochem. J.* (*London*), **42,** 528–31 (1948)
107. Quastel, J. H., and Scholefield, P. G., *Bact. Revs.*, **15,** 1–53 (1951)
108. Lees, H., *Nature*, **158,** 97 (1946)
109. Quastel, J. H., and Scholefield, P. G., *Appl. Microbiol.*, **1,** 282–87 (1953)
110. Baalsrud, K., and Baalsrud, K. S., in *Phosphorus Metabolism*,**II,** 544–76 (W. D. McElroy, and Glass, B., Eds.) (The Johns Hopkins Press, Baltimore, Md., 930 pp., 1952.
111. Bömeke, H., *Arch. Mikrobiol.*, **14,** 271–86 (1948)
112. Meiklejohn, J., *J. Soil Sci.*, **4,** 59–68 (1953)
113. Rittenberg, S. C., and Grady, R. P., *J. Bact.*, **60,** 509–10 (1950)
114. Meiklejohn, J., *Plant and Soil*, **3,** 88–93 (1951)
115. Meiklejohn, J., *Proc. Soc. Appl. Bact.*, **15,** 77–81 (1952)
116. Jensen, H. L., *Nature*, **165,** 974 (1950)
117. Kermack, W. O., and Lees, H., *Science Progr.*, No. 157, 44–53 (1952)
118. Jensen, H. L., and Sörensen, H., *Acta Agric. Scand.*, **2,** 295–304 (1952)
119. Gleen, H., *Nature*, **168,** 117–18 (1951)
120. Vogler, K. G., and Umbreit, W. W., *J. Gen. Physiol.*, **26,** 157–67 (1942)
121. Fujishige, H., and Haga, K., *Acta Phytochim.* (*Japan*), **14,** 141–49 (1944)
122. Nathansohn, A., *Mitt. zool. Sta. Neapel*, **15,** 655–80 (1902)
123. Verhoeven, W., *Aerobic sporeforming nitrate reducing bacteria*, 122–32 (Doctoral thesis, Univ. of Delft, Delft, Holland, 160 pp., (1952)
124. Lees, H., *Biochem. J.* (*London*), **52,** 134–39 (1952)
125. Lees, H., *Nature*, **169,** 156–57 (1952)
126. Hofman, T., and Lees, H., *Biochem. J.* (*London*), **54,** 579–83 (1953)
127. Kluyver, A. J., and Donker, H. J. L., *Chem. Zelle u. Gewebe*, **13,** 134–90 (1926)
128. Quastel, J. H., and Scholefield, P. G., *Nature*, **164,** 1068–72 (1949)
129. Quastel, J. H., Scholefield, P. G., and Stevenson, J. W., *Nature*, **166,** 940–42 (1950)
130. Jensen, H. L., *J. Gen. Microbiol.*, **5,** 360–68 (1951)
131. Quastel, J. H., Scholefield, P. G., and Stevenson, J. W., *Biochem. J.* (*London*), **51,** 278–84; 284–86 (1952)
132. Hutton, W. E., and ZoBell, C. E., *J. Bact.*, **65,** 216–19 (1953)
133. Schlegel, H. G., *Arch. Mikrobiol.*, **18,** 362–90 (1953)
134. Kluyver, A. J., and Manten, A., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **8,** 71–85 (1942)
135. Wilson, E., Stout, H. A., Powelson, D., and Koffler, H., *J. Bact.*, **65,** 283–87 (1953)
136. Schatz, A., *J. Gen. Microbiol.*, **6,** 329–35 (1952)
137. Gest, H., *J. Bact.*, **63,** 111–21 (1952)
138. Schatz, A., Isenberg, H. D., and Trelawny, G. S., *Bact. Proc.*, 88 (1953)
139. Green, M., and Wilson, P. W., *J. Bact.*, **65,** 511–17 (1953)
140. Hyndman, L. A., Burris, R. H., and Wilson, P. W., *J. Bact.*, **65,** 522–31 (1953)
141. Yamagata, S., and Nakamura, H., *Acta Phytochim.* (*Japan*), **10,** 297–311 (1938)
142. Lascelles, J., and Still, J. L., *Australian J. Exptl. Biol. Med. Sci.*, **24,** 37–48 (1946)
143. Wilson, J. B., and Wilson, P. W., *J. Gen. Physiol.*, **26,** 277–86 (1943)
144. Joklik, W. K., *Australian J. Exptl. Biol. Med. Sci.*, **28,** 331–38 (1950)
145 Hofman, T., and Lees, H., *Biochem. J.* (*London*), **52,** 140–42 (1952)

146. Hofman, T., and Lees, H., *Biochem. J. (London)*, **53,** v, 1953
147. Hofman, T., *Biochem. J. (London)*, **54,** 293–95 (1953)
148. Work, E., and Denman, R. F., *Biochim. et Biophys. Acta*, **10,** 183 (1953)
149. Work, E., and Dewey, D. L., *J. Gen. Microbiol.*, **9,** 394–406 (1953)
150. Synge, R. L. M., *J. Gen. Microbiol.*, **9,** 407–9 (1953)
151. Chargaff, E., Zamenhof, S., Brawerman, G., and Kerin, L., *J. Am. Chem. Soc.*, **72,** 3825 (1950)
152. Gandelman, B., Zamenhof, S., and Chargaff, E., *Biochim. et Biophys. Acta*, **9,** 399–401 (1952)
153. Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9,** 402–5 (1952)
154. Franz, I. D., Feigelman, H., Werner, A. S., and Smythe, M. P., *J. Biol. Chem.*, **195,** 423–28 (1952)
155. van Niel, C. B., *Bact. Revs.*, **8,** 1–118 (1944)
156. van Niel, C. B., *Bacterial Photosyntheses*, Chapt. **75,** 1074–88, in *The Enzymes*, (Sumner, J. B., and Myrbäck, K., Eds., **II,** Pt. 2 (Academic Press, Inc., New York, N. Y., 1440 pp., 1952)
157. Gest, H., *Bact. Revs.*, **15,** 183–210 (1951)
158. Larsen, H., "On the microbiology and biochemistry of the photosynthetic green sulfur bacteria," *Kgl. Norske Videnskab. Selskabs, Skrifter*, No. 1, vii plus 199 pp. (1953)
159. Engelmann, T. W., *Pflügers Arch. ges. Physiol.*, **30,** 95–124 (1883)
160. Larsen, H., *J. Bact.*, **64,** 187–96 (1952)
161. Bicknell, A., *Lloydia*, **12,** 183–84 (1949)
162. Bicknell, A. K., *J. Bact.*, **63,** 145–46 (1952)
163. Pringsheim, E. G., *Bact. Revs.*, **13,** 47–98 (1949)
164. Pringsheim, E. G., *Arch. Mikrobiol.*, **19,** 353–64 (1953)
165. Pringsheim, E. G., *Nature*, **172,** 167–68 (1953)
166. Scardovi, V., *Ann. microbiol.*, **4,** 131–72 (1951)
167. Giesberger, G., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **13,** 135–48 (1947)
168. Duchow, E., and Douglas, H. C., *J. Bact.*, **58,** 409–16 (1949)
169. Murray, R. G. E., and Douglas, H. C., *J. Bact.*, **59,** 157–67 (1950)
170. Hutner, S. H., *J. Bact.*, **52,** 213–21 (1946)
171. Hutner, S. H., *J. Gen. Microbiol.*, **4,** 286–93 (1950)
172. Tokuda, S., *Botan. Mag. (Tokyo)*, **62,** 53–56 (1949)
173. Pardee, A. B., Schachman, H. K., and Stanier, R. Y., *Nature*, **169,** 282–83 (1952)
174. Schachman, H. K., Pardee, A. B., and Stanier, R. Y., *Arch. Biochem. and Biophys.*, **38,** 245–60 (1952)
175. Thomas, J. B., *Koninkl. Ned. Akad. Wetenschap. Proc.*, [C]**55,** 207–8 (1952)
176. Foster, J. W., *J. Gen. Physiol.*, **24,** 123–34 (1940)
177. Foster, J. W., *J. Bact.*, **47,** 355–72 (1944)
178. Siegel, J. M., and Kamen, M. D., *J. Bact.*, **59,** 693–97 (1950)
179. Siegel, J. M., *J. Bact.*, **60,** 595–607 (1950)
180. van Niel, C. B., *Cold Spring Harbor Symposia Quant. Biol.*, **3,** 138–50 (1935)
181. van Niel, C. B., in *Photosynthesis in Plants*, 437–95 (Franck, J., and Loomis, W. F., Iowa State Coll. Press, Ames, Ia., 495 pp., 1949)
182. Kamen, M. D., Ajl, S. J., Ranson, S. L., and Siegel, J. M., *Science*, **113,** 302 (1951)
183. Glover, J., Kamen, M. D., and van Gelderen, H., *Arch. Biochem. and Biophys.*, **35,** 384–408 (1952)

184. Tsukamoto, A., *Botan. Mag.* (*Tokyo*), **62,** 159–67 (1949)
185. Tsukamoto, A., *J. Biochem.* (*Japan*), **39,** 339–47 (1952)
186. Gaffron, H., *Biochem. Z.*, **260,** 1–17 (1933)
187. Gaffron, H., *Biochem. Z.*, **275,** 301–19 (1935)
188. Eisenberg, M., *J. Biol. Chem.*, **203,** 815–36 (1953)
189. Elsden, S. R., and Ormerod, J. G., *Biochem. J.* (*London*), **55,** iii (1953)
190. Cutinelli, C., Ehrensvärd, G., and Reio, L., *Arkiv. Kemi*, **2,** 357–61 (1950)
191. Cutinelli, C., Ehrensvärd, G., Reio, L., Saluste, E., and Stjernholm, R., *Arkiv. Kemi*, **3,** 315–22 (1951)
192. Ehrensvärd, G., and Reio, L., *Arkiv. Kemi*, **5,** 327–32 (1953)
193. Larsen, H., *J. Biol. Chem.*, **193,** 167–73 (1951)
194. Calvin, M., *Harvey Lectures, Ser. 46*, 218–51 (1951)
195. Benson, A. A., Bassham, J. A., Calvin, M., Hall, A. G., Hirsch, H. E., Kawaguchi, S., Lynch, V., and Tolbert, N. E., *J. Biol. Chem.*, **196,** 703–16 (1952)
196. Brown, A. H., and Frenkel, A. W., *Ann. Rev. Plant Physiol.*, **4,** 23–58 (1953)
197. Larsen, H., Yocum, C. S., and van Niel, C. B., *J. Gen. Physiol.*, **36,** 161–71 (1952)
198. Wassink, E. C., Katz, E., and Dorrestein, R., *Enzymologia*, **10,** 285–354 (1942)
199. French, C. S., *J. Gen. Physiol.*, **20,** 711–35 (1937)
200. Morita, S., Suzuki, K., and Takashima, S., *J. Biochem.* (*Japan*), **38,** 255–62 (1951)
201. Kohlmiller, E. F., and Gest, H., *J. Bact.*, **61,** 269–82 (1951)
202. Gest, H., and Kamen, M. D., *Science*, **109,** 558–59 (1949)
203. Gest, H., and Kamen, M. D., *J. Bact.*, **58,** 239–45 (1949)
204. Gest, H., Kamen, M. D., and Bregoff, H. M., *J. Biol. Chem.*, **182,** 153–70 (1950)
205. Siegel, J. M., and Kamen, M. D., *J. Bact.*, **61,** 215–28 (1951)
206. Bregoff, H. M., and Kamen, M. D., *Arch. Biochem. and Biophys.*, **36,** 202–20 (1952)
207. Bregoff, H. M., and Kamen, M. D., *J. Bact.*, **63,** 147–49 (1952)
208. Roelofsen, P. A., *On photosynthesis of the Thiorhodaceae* (Doctoral thesis, Univ. of Utrecht, Utrecht, Holland, 127 pp., 1935)
209. Kamen, M. D., and Gest, H., *Science*, **109,** 560 (1949)
210. Lindstrom, E. S., Burris, R. H., and Wilson, P. W., *J. Bact.*, **58,** 313–16 (1949)
211. Lindstrom, E. S., Tove, S. R., and Wilson, P. W., *Science*, **112,** 197–98 (1950)
212. Lindstrom, E. S., Lewis, S. M., and Pinsky, M. J., *J. Bact.*, **61,** 481–87 (1951)
213. Lindstrom, E. S., Newton, J. W., and Wilson, P. W., *Proc. Natl. Acad. Sci. U. S.*, **38,** 392–96 (1952)
214. Kamen, M. D., and Gest, H., in *Phosphorus Metabolism*, **II,** 507–19 (McElroy, W. D., and Glass, B., The Johns Hopkins Press, Baltimore, Md., 930 pp., 1952).
215. Wall, J. S., Wagenknecht, A. C., Newton, J. W., and Burris, R. H., *J. Bact.*, **63,** 563–73 (1952)
216. Newton, J. W., and Wilson, P. W., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **19,** 71–77 (1953)
217. Scardovi, V., *Ann. microbiol.*, **5,** 179–90 (1953)
218. Tarver, H., Tabachnick, M., Canellakis, E. S., Fraser, D., and Barker, H. A., *Arch. Biochem. and Biophys.*, **41,** 1–8 (1952)
219. Vermeulen, D., Wassink, E. C., and Reman, G. H., *Enzymologia*, **4,** 254–68 (1937)
220. Katz, E., and Wassink, E. C., *Enzymologia*, **7,** 97–112 (1939)
221. French, C. S., *J. Gen. Physiol.*, **23,** 469–81 (1940)

222. French, C. S., *J. Gen. Physiol.*, **23**, 483–94 (1940)
223. Duysens, L. N. M., *Transfer of excitation energy in photosynthesis* (Doctoral thesis, Univ. of Utrecht, Utrecht, Holland, 96 pp., 1952)
224. Weigl, J. W., *J. Am. Chem. Soc.*, **75**, 999–1000 (1953)
225. Lascelles, J., *Biochem. J.* (*London*), **55**, iv (1953)
226. Polgár, A., van Niel, C. B., and Zechmeister, L., *Arch. Biochem. and Biophys.*, **5**, 243–64 (1944)
227. Goodwin, T. W., and Osman, H. G., *Biochem. J.* (*London*), **53**, 541–46 (1953)
228. Goodwin, T. W., and Osman, H. G., *Arch. Biochem. and Biophys.*, **47**, 215 (1953)
229. Turian, G., *Helv. Chim. Acta*, **33**, 1988–93 (1950)
230. Turian, G., and Haxo, F., *J. Bact.*, **63**, 690–91 (1952)
231. Volk, W. A., and Pennington, D., *J. Bact.*, **59**, 169–70 (1950)
232. van Niel, C. B., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **12**, 156–66 (1947)
233. Manten, A., *Phototaxis, phototropism, and photosynthesis in purple bacteria and blue-green algae* (Doctoral thesis, Univ. of Utrecht, Utrecht, Holland, 87 pp., 1948)
234. Manten, A., *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **14**, 65–86 (1948)
235. Milatz, J. M. W., and Manten, A., *Biochim. et Biophys. Acta*, **11**, 17–27 (1953)
236. Clayton, R. K., *Arch. Mikrobiol.*, **19**, 107–24 (1953)
237. Clayton, R. K., *Arch. Mikrobiol.*, **19**, 125–40 (1953)
238. Clayton, R. K., *Arch. Mikrobiol.*, **19**, 141–65 (1953)
239. Thomas, J. B., and Nijenhuis, L. E., *Biochim. et Biophys. Acta*, **6**, 317–24 (1950)
240. Thomas, J. B., *Biochim. et Biophys. Acta*, **5**, 186–96 (1950)
241. Thomas, J. B., and Goedheer, J. C., *Biochim. et Biophys. Acta*, **10**, 385–90 (1953)
242. Arkani, M., and Stary, Z., *Folia Pharm.* (*Istanbul*), **2**, 3 (1952); (Cited in Asselineau, J., and Lederer, E., *Fortschr. Chem. org. Naturstoffe*, **10**, 170–273 (1953)
243. Asselineau, J., and Lederer, E., *Fortschr. Chem. org. Naturstoffe*, **10**, 170–273 (1953)
244. Duysens, L. N. M., *Nature*, **168**, 548–50 (1951)
245. Arnold, W., and Oppenheimer, J. R., *J. Gen. Physiol.*, **33**, 423–35 (1950)
246. Schenck, G. O., *Naturwissenschaften*, **40**, 205–12, 229–38 (1953)
247. Calvin, M., *Chem. Eng. News*, **31**, 1622–25, 1735–38 (1953)
248. Hill, R., *Symposia Soc. Exptl. Biol.*, **5**, 222–31 (1951)
249. Hill, R., *Advances in Enzymol.*, **12**, 1–39 (1951)
250. Davenport, H. E., and Hill, R., *Proc. Roy. Soc.* (*London*) [B]**139**, 327–45 (1952)
251. Vernon, L. P., *Arch. Biochem. and Biophys.*, **43**, 492–93 (1953)
252. Elsden, S. R., Kamen, M. D., and Vernon, L. P., *J. Am. Chem. Soc.*, **75**, 6347–48 (1953)
253. Vernon, L. P., and Kamen, M. D., *Arch. Biochem. and Biophys.*, **44**, 298–311 (1953)
254. Elsden, S. R., in *Autotrophic Micro-organisms, 4th Symposium Soc. Gen. Microbiol.*, **4**, 202–23 (1954)
255. Roelofsen, P. A., *Proc. Acad. Sci. Amsterdam*, **37**, 660–69 (1934)
256. Wassink, E. C., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **12**, 281–93 (1947)
257. Duysens, L. N. M., *Nature* (In press)

LEPTOSPIRA[1]

By H. Schlossberger and H. Brandis[2]
Hygienic Institute of the City and University of Frankfurt, Germany

General Considerations

The study of *Leptospira* is a fairly young branch of microbiology. Up to the late thirties it was commonly believed that leptospiroses were rare diseases, but extensive investigations have since shown that they occur in all continents. The various leptospiroses have been clinically differentiated from other diseases [Gsell (1)]. Knowledge of the epidemiology and reservoirs of the leptospiroses has been increased by numerous publications [reviewed by Gsell (1,2), Rimpau (3), and van Thiel (4)]. Marked advances also have been made in the differentiation of individual types: until about 1930 the biological unity of the leptospiras was accepted, but subsequent investigations have proved not only that the water leptospiras can be distinctly differentiated from the pathogenic species but also that within the latter group a number of different types occur. The pathogenic leptospiras can be distinguished by their antigenic structure, and often by their epidemiological and pathological behavior.

About 40 types of pathogenic leptospiras have been described to date [see Table by Schlossberger & Brandis (5)], but it is not known whether all of them represent distinct species or varieties, since comparative investigations have not been sufficiently extensive. Wiesmann (6), in his excellent review on the antigenic properties of the leptospiras, suggests that the known forms be compared and classified as an international undertaking. He believes that by such a procedure the number of definitely differentiable species would be reduced to about fifteen.

Very little is known about the metabolism and the chemical composition of the leptospiras, or about the mechanisms of pathogenesis. Intensive investigations of these matters are still necessary.

The systematic position of the leptospira group.—According to Bergey's *Manual of Determinative Bacteriology*, the genus *Leptospira* Noguchi 1917 belongs to the family *Treponemataceae* which comprises, together with the family *Spirochaetaceae* Swellengrebel 1907, the order *Spirochaetales* [Davis (7)]. The true position of the *Spirochaetales* is not entirely clear. Doflein (8) in 1911 considered them to be related to the blue-green algae; Brumpt (9) in 1936 called them protozoa; Müller (10) and others in 1950 considered them bacteria. Other authors assigned them a place between protozoa

[1] The survey of literature pertaining to this review was completed in December, 1953.

[2] We wish to thank Dr. Joseph Wolfgang Winters for his careful translation of this paper from the original German version.

and bacteria. Recently Schlossberger, Jakob & Piekarski (11), on the basis of electron microscopical and cytological studies, expressed themselves in favor of the bacterial nature of spirochetes. Jakob (12) described a series of "transparent" and "nontransparent" regions in electron micrographs of spirochetes; Schlossberger (13) showed similar dense areas in leptospira with the HCl-Giemsa method of Piekarski-Robinow. It seems likely, therefore, that dark areas in electron micrographs represent nuclear structures, similar to those of bacteria. After aging, heating, or contact with antiserum, leptospiras may show from 4 to 12 granules distributed irregularly throughout the cell [Herreweghe (14)]. The interpretation of dense regions of the cell as nuclear structures must, therefore, be accepted with reservation. Although the lack of a typical cell nucleus would speak for classification of the spirochetes with the bacteria, they show certain morphological features which markedly differentiate them from all other microorganisms. According to the electron microscope studies of Bradfield & Carter (15) all spirochetes possess fibrillae, arranged in the form of a spiral, lying either inside or outside of the cell membrane. Because of these morphological peculiarities Bradfield & Carter feel justified in considering spirochetes as a special group within the kingdom of microorganisms.

Morphology.—The leptospiras are extremely delicate microorganisms with a length of from 10 to 20 μ. Occasionally much larger specimens occur (up to 85 μ). The thickness is from 0.1 to 0.2 μ; Babudieri (16, 17) reports values between 0.06 μ and 0.12 μ. They show from 12 to 18 regular, but very small primary spirals which are recognizable only at very high magnification. The cell shows marked differentiation into one seemingly thicker and more rigid middle piece and two hooklike, bent, pointed end pieces. There are no flagella. Only the dark field microscope permits accurate observation of form and motion.

Uhlenhuth (17a) in 1920 established that the individual species of *Leptospira* do not differ morphologically. Ruska (18), Morton & Anderson (19), Jakob (20), van Thiel (4), Babudieri (16, 17), Wiesmann (6), and Breese and co-workers (21) have reported observations with the electron microscope. After fixation with osmic acid Babudieri (16, 17) could show a central axial thread, with the cytoplasm arranged spirally around it, and a cell membrane. The delicate whiplike structures, with or without terminal bodies, described by Jakob ("leptospirogene"), Babudieri considers dead, degenerated forms in which the axial thread had been liberated as a result of destruction of the membrane and the protoplasm. The existence of a granular stage of development has not been proven. Babudieri (22) could also show that the spermlike forms ("S-Formen") which Jakob saw in electron micrographs of *L. canicola* were contaminants (a mold, *Hyphomicrobium vulgare*) which had nothing to do with *Leptospira*. The axial thread is the most resistant part of the cell according to Babudieri. It persists after autolysis and after the action of pepsin or papain on the cells. However, penicillin (0.1 unit per ml.) lyses the cells quickly and almost completely. Woratz (23) recently reported

spherical structures, which he calls resting forms, in older cultures of *Leptospira*.

Living leptospiras can be observed by phase contrast microscopy, but this method offers no advantage over dark field microscopy. Unstained leptospiras cannot be seen with the ordinary light microscope. Leptospiras stain with difficulty. Useful are Giemsa solution and the stains of Pulcher (6), Vransky (6), and Vago (24). Excellent preparations can be obtained also by the silver method of Fontana-Tribondeau. Cultures in serum-free media give particularly clear pictures in stained and electron micrographs [Wiesmann (6)].

Growth requirements, metabolism, and cultivation.—The most effective medium for the cultivation of leptospiras is that of Korthof (25) with the addition of 10 per cent sterile (preferably unfiltered) rabbit serum. Fletcher's semisolid medium and Vervoort's medium are suitable, too (6, 25a). Addition of yeast extract or hemoglobin does not materially improve the medium. Other animal sera give irregular results. The presence of a body fluid is essential. Brede & Lempfrid (26) achieved good growth with 23 strains, using allantoic fluid of 13-day-old chick embryos instead of serum. The optimum pH lies between 7.3 and 7.5; according to Chang (27) growth is hindered at pH values of 7.8 or 6.8. *L. sejroe* and *L. icterohemorrhagiae* Wismar do not change the pH of Korthof's medium; *L. grippotyphosa* makes the medium more basic [Frunder (28)].

The generation time is shorter at 37°C. than at lower temperatures, but the organisms die off much faster. The most suitable temperature for growth *in vitro* lies between 25° and 28°C. According to Chang (29), *L. icterohemorrhagiae* has a generation time of about 32 hr. Vransky (see 6) assumes that this time lies between 5 min. and more than 1 hr. The virulence of the organisms is usually lost very rapidly on transfer.

Attempts to substitute chemically defined substances for the serum in media have not been very successful. Greene (30) found that amino acids could not replace serum for the growth of *L. canicola* in Schüffner's medium. Schneiderman and co-workers (31) showed later that growth is increased by addition (mg. per cent) of L-arginine-HCl (40 to 150), L-asparagine (20 to 200), L-aspartic acid (60 to 100), L-glutaminic acid (40 to 500), or proline (100 to 500) to the basic salt medium with rabbit serum albumin. Other amino acids, however, were inhibitory. The rabbit serum albumin could not be replaced by a mixture of the amino acids found in albumin. The authors assume, therefore, that albumin as such is essential for growth. Yoshida (32) showed that, among the vitamins, nicotinic acid was a stimulating factor for the growth of *L. icterohemorrhagiae* and *L. hebdomadis*. Schneiderman and co-workers found that thiamine chloride in concentrations of 1 to 200 mg. per cent stimulated growth of *L. canicola*. They were able to grow *L. icterohemorrhagiae*, *L. ballum*, *L. sejroe*, and *L. canicola* for 14 weeks in a medium consisting of asparagine, Ringer's solution, Sörensen buffer, rabbit serum albumin, and thiamine. Gram & Schlipköter (33) confirmed the stimulating

influence of nicotinic acid, and also found nicotinamide, biotin, aneurin, and cocarboxylase to be active. The role of these vitamins in the metabolism of *Leptospira* is not yet known. Further data on the influence of vitamins and amino acids on the growth and respiration of leptospiras are given by Geiman (34).

L. icterohemorrhagiae can be cultivated in the chick embryo (35). Multiplication takes place mostly in the liver but also in the allantoic fluid, in the blood, and in the vitelline membrane (36). Embryos in any stage can be infected and usually die on the fourth day after inoculation.

Cultivation on a solid medium has not been achieved. However, Zironi & Carlinfanti (37) observed the movement of *Leptospira* on the surface of solid media. Woratz (38) believes he obtained growth on a solid medium consisting of Korthof's medium, 1.5 to 2 per cent agar and 10 per cent whole rabbit blood. "Colonies" developed in tightly-stoppered tubes only after two months and consisted of flat, needlelike structures about 2 mm. long. A network of such needles formed in about six months. Cultures of *Leptospira* supposedly remain viable on this medium for over a year without subculturing. The structure of *Leptospira*, as seen in liquid cultures, is not discernible in microscopic preparations of these "colonies". Confirmation of these reports is still lacking. Lawrence (39), working with a semisolid medium, found that aerobic *Leptospira* will grow in an oxygen range of 0.5 to 90 per cent. 2,6-Dichlorphenol-indophenol is reduced and makes a good indicator of growth. *Leptospira* remain viable for more than a year in Korthof's medium [Babudieri (40)]; *L. icterohemorrhagiae* retains its virulence for over 2½ years if stored at −70°C. [Weinman & McAllister (41)].

Antigenic properties of leptospiras and serological diagnosis of leptospiroses. —Since the leptospiras cannot be differentiated culturally or morphologically, differentiation must rest, as Schüffner suggested, on antigenic structure, host range, geographical distribution, behaviour in experimental animals, and disease symptoms. There is no agreement as to whether serologically different strains should be designated as species or types. If we were to observe the international nomenclature code we should, as Wiesmann (6) stresses, talk about species within the genus *Leptospira*. Wolff & Broom (42), on the other hand, suggest that strains of identical antigenic structure be designated as serotypes. Serologically related serotypes are to be grouped into "serogroups." The following definition of a *Leptospira* serotype was provisionally adopted in 1953 (see 42):

> Two strains are considered to belong to different serotypes if, after cross-absorption with adequate amounts of heterologous antigen, 10 per cent or more of the homologous titer regularly remains in each of the two antisera.

An adequate amount of antigen is defined as

> a quantity which removes from the antiserum all antibodies which react with the strain used for the absorption. It is recommended that the original homologous titers of antisera used for absorption should neither greatly exceed nor fall short of 1/3,000.

The difficulty in the serological typing of leptospiras lies in their complex antigenic structure, about which little is known. Schlossberger, Grillo & Scheele (43) were the first to point out that individual strains are composed of several antigens. The distribution of these antigens in different strains is unequal. Gispen & Schüffner (44) proved the existence of two "biotypes" of *L. icterohemorrhagiae*, the "complete" type possessing two antigens, A and B, the "incomplete" type having only the A antigen. Individual strains of *L. icterohemorrhagiae* also show other antigenic deviations. Thus, sera of patients with Weil's disease or immune sera of rabbits frequently react with some strains of *L. icterohemorrhagiae* and not with others. Furthermore, such sera may agglutinate other types, such as *L. canicola*. Since strains of *L. icterohemorrhagiae* also react with anti-canicola sera, Schlossberger maintains that there is a close serological relationship between these types, and that in many cases no sharp borderline exists. Vaucel (45) also found a serological relationship between *L. icterohemorrhagiae* and *L. autumnalis*. Schlossberger (46) showed the close relationship of Leptospira in yet another way by achieving a "type transformation" through directed mutation. By simultaneous injection of a culture of *L. icterohemorrhagiae*, avirulent for guinea pigs, together with killed cells of a freshly cultured Saxkoebing strain into the peritoneal cavity of a guinea pig it was possible to transform the former into the Saxkoebing type.

Rimpau (3) divides leptospiral antigens into principal and secondary antigens. The principal antigens predominate and determine the type. The principal antigen of one type may appear as a secondary antigen in another type. This distribution of antigens explains the overlapping observed in reactions between monovalent immune sera and heterologous types. These side reactions, noticeable in the agglutination-lysis-test or in the complement fixation reaction, create considerable difficulties in the differentiation of types or in the serological diagnosis of the disease. Despite this limitation, the agglutination-lysis-test remains the only usable means for type differentiation. The reliability of differentiation on grounds of epidemiological peculiarities, of geographical distribution, of animal pathogenicity, or of the clinical picture is still questionable. It has happened repeatedly that types such as *L. grippotyphosa* or *L. pomona*, originally assumed to be confined to a certain animal host and a narrowly defined geographical area, have been found later in other host species and entirely different areas. Furthermore, serologically identical strains may elicit different diseases in man according to the geographic area. Thus, in Indonesia *L. bataviae* produces a severe and often fatal infection accompanied by icterus, while apparently the same organism produce a benign, nonicteric disease in Silesia or Italy. Conversely, identical disease symptoms in man may be elicited by serologically different types.

The antigenic structure of individual strains is generally assumed to be constant. Results reported by Bessemans *et al.* (47, 48, 49), which indicate a permanent antigenic variation, are, therefore, of special interest. These authors used a nonpathogenic strain (Gd IV) isolated from the tap water of

Gent, and serologically closely related to *L. icterohemorrhagiae*. In the first experiments (47) strain Gd IV was exposed to homologous antiserum seven times for 24 hr. during a period of five months. The intermediate passages were made in Vervoort-medium No. 2 containing immune serum in low concentration. A variant (Gd IV-1) isolated after this treatment differed markedly from the original strain in the agglutination-lysis-test and has retained its newly-acquired properties for over eight years. Bessemans (48) also did control experiments with strains originating from single cells isolated with the aid of a micromanipulator. The results were the same. This variation, therefore, is not attributable to the selection of a pre-existing serological variant by the immune serum. The first variant obtained in the experiments with the micromanipulator (Gd IV-M 1) could be transformed by contact with homologous antiserum into a second variant (Gd IV-M 2), which in turn was transformed into a third variant (Gd IV-M 3). Each of these three serological variants reacted strongly with its homologous antiserum but only weakly with heterologous sera. One must assume that a permanent, stable change in antigenicity had occured. Bessemans (49) concludes that serological mutations might possibly occur spontaneously, and he cautions against classifying the leptospiras into too many serological types. The observations made with Gd IV should, however, not be generalized. Bessemans himself (49) did not succeed in adapting *L. icterohemorrhagiae* "Wijnberg" to its own antiserum. In this connection Babudieri's studies (50, 51) on the antigenic structure of water leptospiras are of interest. This author compared the agglutinability of 34 strains and found that they could be grouped into four main serological groups. Absorption experiments showed the existence of apparently incomplete antigens capable of eliciting antibodies in the body but incapable of reacting with them *in vitro*.

The types of *Leptospira* can be grouped partly according to their antigenic relationships.

Wiesmann (6) suggested the classification shown in Table I. *L. australis*, *L. mitis* (= *L. hyos*), *L. autumnalis*, and *L. bataviae* are independent serological types. Borg-Petersen (52) devised a diagnostic scheme for the hebdomadis group, designating with the Roman numeral I an antigen that occurs in all types of this group, while antigens 2 to 6 are specific for the individual types. The types and their antigens are shown in Table II.

Little is known about the chemical composition of leptospiral antigens. By disrupting cells of *L. icterohemorrhagiae* "Wijnberg" with an equal volume of a solution containing 0.1 M NaCl, 1.2 M sodium citrate, and 0.4 per cent sodium desoxycholate, Schneider (52a) succeeded in isolating an immunologically-reactive heat-stable genus-specific complement-fixing nondialyzable substance in a mixture with a nondialyzable polynucleotide moiety. The active principle appears to be a pentose. Starting from the crude cell protein remaining after this extraction, Schneider (52b) found three additional nondialyzable pentose-containing, complement-fixing materials.

The identification of newly-isolated strains is done mostly by the agglu-

TABLE I

SEROLOGICAL CLASSIFICATION OF LEPTOSPIRA*

Group	Composed of
1. Grippotyphosa group	*L. grippotyphosa* *L. bovis* (Palestinense) *L. geffeni*†
2. Pomona group	*L. pomona* *L. bovis* (New York)
3. Hebdomadis group	*L. hebdomadis* *L. sejroe* *L. saxkoebing*
4. Canicola group‡	*L. canicola* *L. salinem* *L. ballum*

* As suggested by Wiesmann (6).
† Added by the present authors.
‡ *L. icterohemorrhagiae* is also related to this group.

tination-lysis-test with sera against known types. Cross agglutination tests of these types with immune serum against the new strain should follow. As in the case of salmonellae, absolute titration values are not always indicative. The identity or nonidentity of antibodies must be shown. This can be done only with aid of Castellani's absorption test although considerable difficulties are encountered, since it is hard to obtain sufficient quantities of leptospiras. Furthermore, Wolff (53) obtained different results, employing techniques in the cross absorption test. Results of the absorption test obtained in different laboratories, therefore, can not be compared without reservation. Blindenhöfer (54) attempted type differentiation employing Pfeiffer's test in rats.

Since the isolation of leptospiras from human blood is possible only in the

TABLE II

SEROLOGICAL DIFFERENTIATION OF THE HEBDOMADIS GROUP

Species	Antigens
L. hebdomadis	I, 2
L. H.C.	I, 3
L. 3705	I, 4
L. sejroe	I, 5
L. saxkoebing	I, 6

first few days of the disease, and even then not regularly, diagnosis relies mainly on the serological demonstration of leptospiral antibodies. This can be accomplished by the Rickenberg phenomenon (55), the complement fixation reaction, or the agglutination-lysis-test. The usefulness of the thrombocytobarin test for practical diagnostic purposes has not yet been established [van Thiel (4)]. The advantage of the complement fixation reaction lies in the stability of the antigen and in the fact that the test can be carried out by anyone who has mastered the Wassermann technique (4). Instead of the liver extracts from infected man or guinea pig originally employed ,the test is carried out today with extracts of leptospiras (56, 57). Randall and co-workers (58) use formalinized suspensions which had been exposed to sonic vibration for 10 min. at 9400 c.p.s. These authors state that differentiation between Weil's disease and canicola fever is not possible with the antigens obtained from the respective organisms since both antigens exhibit marked overlapping reactions. The complement fixation reaction usually gives lower titers than the agglutination-lysis-test. This latter reaction becomes positive at an earlier stage [between 4 and 13 days (59)], and it is more type specific.

The titer in the agglutination-lysis-test depends largely on the technique and the experience of the investigator. Borg-Petersen & Fagraeus (60), employing living cultures, found an inverse ratio between the titer and the density of the suspension. Variation in sensitivity of individual strains of *Leptospira* can be observed. For these reasons the results from individual laboratories are not comparable. Lübbers (61) reported that examinations of sera carried out in four different laboratories showed markedly different titers. Borg-Petersen (60) suggests, therefore, a standardization of the agglutination-lysis-test (see also 61a). An agglutination test also can be carried out with formalinized cultures, which have the advantage of greater stability but are not lysed by immune sera. Stoenner (61b) describes a macroscopic capillary tube agglutination test. The antigen is prepared by suspending formalin-killed selected strains of *Leptospira* in hypertonic buffered sodium chloride solution. The test is specific but gives more cross-reactions than the microscopic agglutination lysis test with living cultures. There are also methods for macroscopic slide-agglutination (see 61c). The number of leptospira types used in the agglutination-lysis reaction depends on the epidemiological conditions. The following types are now routinely used in our institute: *L. icterohemorrhagiae* (three strains); *L. canicola* (two strains); *L. grippotyphosa* (two strains); *L. sejroe; L. saxkoebing; L. australis* A; *L. pomona; L. autumnalis; L. mitis* (=*L. hyos*); and *L. bataviae.* In view of the discrepancies in technique and in individual results the values considered diagnostically significant vary from one laboratory to another. Essefeld (see 60) considers 1:300, Wiesmann (6), 1:400, Rimpau (3) and Broom (59), 1:1000 to be a titer indicative of the disease. In the beginning of the disease, titers for the heterologous types may be equally high or even higher than those for the homologous leptospiral type [Führer (62)].

Wolff and co-workers (63) describe a case history of a lethal canicola infection. On the day of death (eleventh day of the disease) the serum had a titer of 1:3000 for both *L. icterohemorrhagiae* and *L. canicola*. Only by absorption tests could it be established that *L. canicola* was the causative agent. Frequently, however, the titers of the homologous type rise considerably during the course of the disease and are markedly above the titers of the heterologous types. The interpretation of the "heteroagglutinations" is difficult since there appears to be great variation between individual patients. Wiesmann (6) differentiates between specific and nonspecific "heteroagglutination." The formation of specific heteroagglutinins is attributable to the complex antigenic structure of the leptospiras. The occurrence of nonspecific antibodies, whose formation is unexplained, and for which there are no equivalent antigens, depends mainly on variations between host species or even within the same host species (6). Nonspecific heteroagglutinins are, for instance, rarely found in the rabbit, while they frequently occur in man or guinea pigs. Hence, the rabbit is especially useful for the study of serological relationships.

Weil's Disease

Donatien & Gayot (64) studied the experimental infection in the guinea pig. The animals are susceptible to organisms introduced by intraperitoneal, subcutaneous, or intranasal inoculation and by feeding or by scarification of the skin. The infection is not transmitted by mere contact of normal with infected animals. While guinea pigs infected with virulent strains die within four to seven days, animals infected with laboratory strains often show no external symptoms. Zanussi & Squadrini (65) infected guinea pigs with the Wijnberg strain which elicits no visible symptoms. At autopsy, up to 50 days after inoculation, degenerative changes of the kidney and liver as well as hemorrhages (especially of the lungs) were found. Thus, mild and nonlethal infections may elicit distinct histologically-recognizable changes. Virulent leptospiras may travel through the placenta of the guinea pig and infect the fetus (6, 66). Maroccan squirrels (*Atlantoxerus getulus*) are also susceptible to *L. icterohemorrhagiae*; 22 such animals, infected with virulent material, died within three to seven days (67). The animals showed lung infarcts and, in part, icterus. Field & Sellers (68) observed infections with L. icterohemorrhagiae in four one-month-old piglets, one of which succumbed to the infection. All animals had fever and icterus. The same authors (69) also report a lethal infection of a 14-day-old calf. Anchezar and co-workers (70) describe the occurrence of *L. icterohemorrhagiae* (synonymous with their *L. bonariensis*) in the swamp beaver of the zoological garden in Buenos Aires. Frogs and fish play no part in the epidemiology of Weil's disease. Leptospiras die off in fish (*Leuciscus rutilus, Cyprinus carassius*) within a few days (4). *L. icterohemorrhagiae* can be transmitted experimentally through *Ornithodorus moubata*. Schlossberger & Langbein (71) showed that the organisms survive in the tick for at least 39 days. Normal guinea pigs can be infected with these

ticks. The leptospiras find their way into the eggs of the ticks and thus into the next generation.

The incidence of natural infection in rats has been reported by a number of workers (72 to 81) as being between 14 and 60 per cent. The number of positive findings depends largely on the method of investigation. The best results are obtained when direct dark field observations of the kidney are carried out together with cultivation, guinea pig inoculation, and histological studies. Young rats are more frequently free of *Leptospira* than are adult animals (80, 82).

Human infections occur largely as a result of contact with infected water. Boyer and co-workers (83) studied 164 cases of Weil's disease which occurred between 1943 and 1951 in the province of the Seine; 46.9 per cent of the patients acquired the infection by bathing in infected water, 8 per cent were fishermen, and in 30.5 per cent the disease had an occupational origin (slaughterhouse workers, etc.). Of all patients, 92.7 per cent were male. In children and older persons infection was rare. There was a marked seasonal peak, culminating in August. Summaries on the occurrence of Weil's disease can be found in Gsell (1), Molner, Meyer & Raskin (84), Broom & Alston (85, 86, 87), Borg-Petersen (88), and Wolff & Ruys (88a). Goudie and co-workers (89) report on the incidence of antibodies against *L. icterohemorrhagiae* in fishmongers. In one series of 210 individuals they found antibodies in 24.2%, and in a second series, three positive individuals among 162.

Canine Leptospirosis

L. canicola.—In contrast to its role in the epidemiology of *L. icterohemorrhagiae*, the rat does not take part in the infectious cycle of *L. canicola* (90). The dog is the principal host of this species. Guinea pigs are only slightly susceptible, young animals occasionally succumbing to a canicola infection with icteric symptoms, especially if several passages are carried out (91). Three- to four-week-old golden hamsters can be infected regularly. These animals die within a few days (91, 92, 93). Among the typical symptoms in dogs are vomiting, rise in temperature, uremia, and anuria. Mortality is 50 per cent. Subacute and subclinical infections which frequently occur are much harder to recognize (94, 95). In these cases the organisms may be excreted for months, perhaps years, from numerous foci in the kidneys (96). Transmission occurs by direct contact with the urine or through water. Canicola infections occur largely in young animals. Male dogs are affected more frequently than females. Van Riel and co-workers (97) studied experimental infection with strain "Cureghem" in puppies three months old over a period of 12 months. Three animals died; others showed hardly any clinical symptoms. The antibody titers rose from 1:10,000 to 1:30,000 and fell after several months to 1:300. The excretion of leptospiras was intermittent and ceased during the winter months. There is apparently a great variation in the virulence of *L. canicola* strains and in the susceptibility of dogs. The normal inci-

dence of *L. canicola* in dogs was studied by many workers, positive titers being found in from 10 to 50 per cent of the dogs (91, 96, 98,to 105). The disease usually occurs sporadically in dogs, but occasional mass infections are observed (91, 106, 107, 108). Human infections are apparently not as rare as was previously assumed. Minkenhof (109) reports 98 cases found in the world literature up to 1947. Since then, human canicola infections have been reported by many authors (1, 110, 111), 103 human canicola infections being diagnosed serologically in our Institute between 1947 and 1952. Among these were 50 men and 44 women, the source of nine sera being unknown. There was a canicola epidemic in Frankfurt in the fall of 1947 with at least 47 cases [Wirtz (112)].

Other canine leptospira.—Besides *L. canicola* one finds mainly *L. icterohemorrhagiae* in dogs, but other types of leptospiras may also be found. In a serological survey of 290 dogs, three-fourths of which had disease symptoms, Steigner (99) found 112 with a positive canicola titer (1:500), 4 with a positive titer for *L. grippotyphosa*, 1 each with a significant titer for *L. icterohemorrhagiae*, *L. sejroe*, and *L. pomona* [see also Schermer (113)]. Between 1949 and 1952 we found 238 dog sera in Frankfurt with positive agglutination reactions. The serum titer indicated the following leptospiral infections: 38 with *L. icterohemorrhagiae*, 173 with *L. canicola*, 2 with *L. grippotyphosa*, 5 with *L. sejroe*, 18 with *L. saxkoebing*, and 2 with *L. pomona*. In one case we succeeded in isolating *L. saxkoebing* from the urine. In tropical East Asia, canicola infections in dogs are apparently rarer than other leptospiroses. Mochtar & Collier (114) found only one positive serum for *L. canicola* among the sera of 152 healthy dogs in Djakarta (Java). Fifty-six sera, on the other hand, reacted with *L. bataviae*, *L. javanica*, or *L. icterohemorrhagiae*. Mochtar (115) reported that there are four types of leptospiras in dogs in Java, namely, *L. autumnalis*, *L. ballico*, *L. pomona*, and *L. bataviae*. The predominant types in dogs in Sumatra are *L. hebdomadis* and *L. medanensis* [Wolff *et al.* (116)]. The same authors report the first isolation of *L. canicola* from leptospirosis in a dog in Indonesia (116). In sera from 102 dogs in Shizuoka (Japan) Yamamoto (117) found positive agglutination with the following types: *L. icterohaemorrhagiae* [8 sera], *L. canicola* [9], *L. hebdomadis* [7], and *L. autumnalis* [6]. Van Riel (118) serologically diagnosed infections with *L. butembo*, *L. ndambari*, *L. kabura*, and *L. kamituga* in dogs in the Belgian Congo. Whether these newly described types constitute independent species cannot be stated, since comparative investigations are still lacking.

Other leptospira.—Because of lack of space the numerous observations on other types of leptospiras will not be discussed here, save for a few references to the literature. Schüffner & Bohlander (119, 120), Rimpau (3), Gsell & Rimpau (121), Wagener & Mitscherlich (see 1) discuss the incidence of *L. grippotyphosa* in field mice. Whether *L. grippotyphosa* exists for a long time or undergoes exogenous multiplication [as Kathe assumes (122)] has not been established. During an extensive fever epidemic among pea pickers

in Northern Germany, Popp (123) found 80 per cent of the mice in the epidemic area to be infected with *L. grippotyphosa*. Kathe (124) carried out studies with white mice.

Bernkopf (125) describes *L. bovis* as the causative agent of leptospirosis in cattle in Palestine. A number of human infections were also reported. The symptoms were similar to those in Weil's disease (125 to 128). Bernkopf and co-workers (129) and Wolff & Bohlander (130) carried out comparative studies on *L. bovis* and *L. grippotyphosa*. *L. bovis*, according to the latter authors, is not an independent species but belongs, serologically at least, to the *L. grippotyphosa* group. *L. bovis* differs serologically from the New Jersey bovine strain [Bernkopf & Little (131)]. *L. bovis* is nonpathogenic for white mice, guinea pigs, and rabbits, but posesses high virulence for calves (129). Sheep, goats, and monkeys also are susceptible to *L. bovis*. Experimental infection of calves was studied by Ungar & Bernkopf (132). Borg-Petersen (133) carried out 35 passages of *L. bovis* in guinea pigs. Only one animal succumbed to the infection. The organisms could be found in the peritoneal exudate for only a few days. *L. geffeni*, isolated from *Microtus guentheri* and from human infections in Palestine, is serologically related to *L. grippotyphosa* [Olejnik & Shneyerson (134)].

Two strains isolated from cattle in North America, New Jersey bovine strain C 164 and New York bovine strain A, are said to belong to the *L. pomona* group (135). *L. pomona* is also the cause of leptospirosis in Argentina and Australia (136, 137). There are apparently slight antigenic differences between the European and the American type. Serological studies showed that cattle can also be infected with other types [e.g., *L. icterohemorrhagiae*, *L. mitis*, *L. australis*, *L. grippotyphosa*, *L. sejroe* (52, 130, 138, 139)]. However, these seem to be mostly latent infections. The same seems to be true of leptospiroses in horses (for description of acute infections see 139a, b). Periodic eye inflammation of the horse must be regarded as the result of a usually unnoticed leptospiral infection (140 to 143). Schlossberger & Schoop (144) believe that there is a possible connection between the so called "Einschuss" in the horse and a leptospiral infection.

L. pomona is widely distributed in hogs but can also be found in cattle, horses, and dogs, and, more rarely, in rats. Human infections occur largely in certain vocational groups such as swine herders and butchers ["swine herd disease" (see Gsell 1, 145)]. The infection in hogs frequently shows no symptoms (1, 146, 147). Various workers (148 to 154) have made serological studies in hogs. Schmid & Giovanella (155) carried out experimental infection with hogs, three out of six infected animals showing a slight temperature increase, conjunctivitis, and loss of appetite after three weeks. One animal suffered extreme weakness. Five animals survived the infection, one succumbing to swine erysipelas. The agglutination titers were positive on the eleventh day after inoculation, with a maximum value of 1:16,000. One year after inoculation two animals still had titers of from 1:500 to 1:1000. Excretion of the organisms in the urine began in the third week of the infection. Up to six months a great number of leptospiras were excreted. Very few organisms

were found in the urine after one year. The organisms remained motile in urine for 48 hr. at room temperature.

L. pomona is barely virulent for guinea pigs, but excretion in the urine may occur [van Thiel (4)]. Savino & Rennella (156) isolated an organism from pigs, cattle, and horses in Argentine which they designated as *L. suis*. It is identical with *L. pomona*.

L. mitis Johnson occurs in hogs and cattle. Up to 1947, 12 human infections with *L. mitis* had been observed in Queensland. Gsell & Wiesmann (157) described mitis infections in Switzerland in 1948. Babudieri (158) showed in 1951 that *L. mitis* is identical with *L. hyos* described by Savino & Rennella in 1944.

L. ballum was isolated (159) from *Mus musculus spicilegus* in Denmark. It occurs in oppossum and house mice in the United States. This type, antigenically related to *L. canicola*, is virulent for guinea pigs but rarely causes icterus. The first human infection was described by Wolff, Bohlander & Ruys in 1949 (160). Cases were recently detected among rice workers of the Rhone delta (France) and in Spain (160a).

L. saxkoebing was first isolated in 1942 by Borg-Petersen from *Apodemus flavicollis* in Denmark. Schlossberger (see Lübbers, 161) isolated this type from a dog urine in 1949. He also proved the presence of *L. saxkoebing* in the Frankfurt area by isolation from wood mice in 1953. The species is also found in Italy [Babudieri (162)]. Virulence for guinea pigs differs in individual strains. Experimentally infected rats and mice show no symptoms but become excreters (52).

Distribution of leptospiras.—Rimpau (163) has described in detail the distribution of leptospiras in man and animals in different countries. Table III, which summarizes the results of several surveys, shows the distribution of leptospiral types, the presence of which has been established in part by direct cultivation, in part only indirectly by the demonstration of antibodies.

Serum Therapy and Vaccination

Serum therapy can be quite successful when potent, monovalent animal sera are used at an early stage. This therapy has proved its value in Weil's disease as well as in canicola fever and field fever. Active vaccination methods, using heat-killed or formalinized cultures or lyophilized antigens, still need extensive investigation (111, 178 to 182).

Effect of Antibiotics on Leptospira

Penicillin (0.5 units per ml.) has a pronounced *in vitro* action on several types, but its effectiveness in the experimental infection of guinea pigs with *L. icterohemorrhagiae* is doubtful (183). It is not possible to free the animals of leptospiras. Borg-Petersen (184) found organisms in the kidneys of penicillin-treated guinea pigs after one to three months. Heilman (185) found chlortetracycline (aureomycin) effective in the treatment of experimentally-infected golden hamsters. Brunner & Meyer (111, 186) and Uhlenhuth &

TABLE III

DISTRIBUTION OF LEPTOSPIRA

	Types found to date*
Germany (163)	*L. icterohemorrhagiae, L. canicola, L. grippotyphosa, L. sejroe, L. australis., L. pomona, L. bataviae, L. saxkoebing*
France (163)	*L. icterohemorrhagiae, L. canicola, L. grippotyphosa, L. pomona, L. hyos (=L. mitis), L. ballum*
Denmark (164)	*L. icterohemorrhagiae, L. canicola, L. sejroe, L. grippotyphosa, L. bataviae, L. poi, L. ballum, L. saxkoebing*
England (85)	*L. icterohemorragiae, L. canicola*
Italy (165, 166)	*L. icterohemorrhagiae, L. canicola, L. bataviae, L. pomona, L. grippotyphosa, L. sejroe, L. saxkoebing, L. poi, L. pyrogenes, L. mitis*
Switzerland (6, 167)	*L. icterohemorrhagiae, L. canicola, L. sejroe, L. australis A., L. pomona, L. hyos (=L. mitis), L. autumnalis*
Israel (168)	*L. icterohemorrhagiae, L. canicola, L. bovis, L. geffeni, L. pomona*
Dutch East Indies and adjoining territories (169, 170)	*L. andaman, L. australis, L. autumnalis* (2 biotypes), *L. bataviae* (2 biotypes), *L. benjamini, L. cynopteri, L. djasimani, L. grippotyphosa, L. hebdomadis, L. icterohaemoglobinuriae, L. icterohemorrhagiae* (2 biotypes), *L. javanica, L. kebleri, L. medanensis, L. naami, L. pomona, L. pyrogenes, L. sarmini, L. schüffneri, L. sejroe, L. semaranga, L. sentoti, L. vauceli, L. wolffi*
Southern Viet-Nam (171)	*L. icterohemorrhagiae, L. canicola, L. bataviae, L. australis*
Japan (172)	*L. icterohemorrhagiae, L. australis. L. autumnalis, L. hebdomadis, L. canicola, L. pyrogenes*
Australia (173, 173a)	*L. australis, L. pomona, L. mitis, L. icterohemorrhagiae, L. canicola, L. pyrogenes*
North America (174, 174a, b, c)	*L. icterohemorrhagiae, L. canicola, L. pomona* (New Jersey strain), *L. bataviae, L. autumnalis, L. grippotyphosa, L. ballum, L. pyrogenes, L. hebdomadis*
South America (Argentina) (175, 176, 177)	*L. icterohemorrhagiae* (*L. bonariensis*), *L. canicola, L. pomona* (*L. suis*), *L. hyos* (*L. mitis*)
Africa (Belgian Congo) (118)	*L. icterohemorrhagiae, L. bataviae, L. butembo*†, *L. kabura*†, *L. grippotyphosa* (*L. bovis?*), *L. kamituga*†, *L. ndambari*†, *L. bafani*†, *L. benjamini*

* The listings are not made according to frequency of occurrence.

† Not yet compared with known standard types.

Schoenherr (187) successfully treated canicola infections in hamsters with chlortetracycline and prevented the chronic excretion of leptospiras in the urine. A dosage of 25 mg. per kg. body weight, given as late as 40 hr. after inoculation, prevented death of the animals. Oxytetracycline (terramycin) also appears to be effective. The susceptibility of individual strains to antibiotics varies (188), laboratory cultures being more resistant than freshly isolated strains (189). Combinations of several antibiotics do not increase the therapeutic effect. Schlipköter & Gram (190) noted that atabrine, given alone, does not influence the course of canicola infection in hamsters, but that it increases the efficiency of chlortetracycline. Gädeke & Schoenherr (191) showed that canicola-infected hamsters survive after serum treatment but show histological changes in the liver and kidneys at autopsy, while oxytetracycline-treated animals appear normal.

LITERATURE CITED

1. Gsell, O., *Leptospirosen* (H. Huber, Bern, Switzerland, 323 pp., 1952)
2. Gsell, O., *Ergeb. inn. Med. u. Kinderheilk.*, **1**, 367 (1949
3. Rimpau, W., *Die Leptospirose* (monographien *Med. Klin.*, **8**) (Urban & Schwarzenberg, München-Berlin, Germany, 157 pp., 1950)
4. van Thiel, P. H., *The Leptospiroses* (Universitaire Pers, Leiden, The Netherlands, 231 pp., 1948)
5. Schlossberger, H., and Brandis, H., *Klin. Wochschr.*, **28**, 625 (1950)
6. Wiesmann, E., *Ergeb. Hyg. Bakteriol. Immunitätsforsch. u. Exptl. Therap.*, **27**, 323 (1952)
7. Davis, G. E., *Ann. Rev. Microbiol.* **2**, 305 (1948)
8. Doflein, F., *Probleme der Protistenkunde*, **II**, *die Natur der Spirochäten* (Gustav Fischer, Jena, Germany, 36 pp., 1911)
9. Brumpt, E., *Précis de Parasitologie*, 5th ed. (Masson & Cie, Paris, France, 2139 pp., 1936)
10. Müller, R., *Medizinische Mikrobiologie*, 4th. ed. (Urban & Schwarzenberg, Berlin-München, Germany, 542 pp., 1950)
11. Schlossberger, H., Jakob, A., and Piekarski, G., *Naturwissenschaften*, **37**, 186 (1950)
12. Jakob, A., *Klin. Wochschr.*, **24, 25,** 882 (1946–47); **27,** 364 (1949)
13. Schlossberger, H., *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, **155,** 75* (1950)
14. Herreweghe, E., *Acta Biol. Belg.*, **3–4,** 245 (1943)
15. Bradfield, J. R. G., and Carter, D. B., *Nature*, **169,** 944 (1952)
16. Babudieri, B., *Rend. ist. super. sanità*, **11,** 1046 (1948)
17. Babudieri, B., *J. Hyg.*, **47,** 390 (1949)
17a. Uhlenhuth, P., and Zuelzer, M., *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, **85,** 141* (1921)
18. Ruska, H., *Arch. ges. Virusforsch.*, **2,** 480 (1941)
19. Morton, H. E., and Anderson, T. E., *J. Bact.*, **45,** 143 (1943)
20. Jakob, A., *Med. Klin. Munich.*, **42,** 22 (1947)
21. Breese, S. S., Jr., Gochenour, W. S., Jr., and Yager, R. H., *Proc. Soc. Exptl. Biol. Med.*, **80,** 185 (1952)
22. Babudieri, B., *Rend. ist. super. sanità*, **13,** 580 (1950)
23. Woratz, H., *6th Intern. Congr. Microbiol.*, (Rome, Italy, September, 1953)

24. Vago, G., *Arch. Hyg. u. Bakteriol.*, **136,** 261 (1952)
25. Korthof, G., *Zentr. Bakteriol. Abt. I Orig.*, **125,** 429 (1932)
25a. Gochenour, W. S., Jr., Yager, R. H., Wetmore, P. W., and Hightower, J. A., *Am. J. Public Health*, **43,** 405 (1953)
26. Brede, H. D., and Lempfrid, H., *Klin. Wochschr.*, **29,** 671 (1951)
27. Chang, S. L., *J. Infectious Diseases*, **81,** 28 (1947)
28. Frunder, M. A., *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, **158,** 519 (1952)
29. Chang, S. L., *J. Infectious Diseases*, **81,** 35 (1947)
30. Greene, M. R., *J. Bact.*, **50,** 39 (1945)
31. Schneiderman, A., Greene, M. R., Schieler, L., McClure, L. E., and Dunn, M. S., *Proc. Soc. Exptl. Biol. Med.*, **82,** 53 (1953)
32. Yoshida, N., *Fukuoka Acta Med.*, **32,** 88 (1939)
33. Gram, H. G., and Schlipköter, H. W., *Arzneimittel-Forsch.*, **7,** 97 (1953)
34. Geiman, Q. M., *Ann. Rev. Microbiol.*, **6,** 299 (1952)
35. Hallauer, C., and Kuhn, H., *Z. Hyg. Infektionskrankh.*, **124,** 125 (1943)
36. Pumarola Busquets, A., *Laboratorio, Granada*, **4,** 201 (1947)
37. Zironi, A., and Carlinfanti, E., *Boll. ist. sieroterap. milan.*, **20,** 51 (1941)
38. Woratz, H., *Z. Hyg. Infektionskrankh.*, **134,** 78 (1952)
39. Lawrence, J. J., *Australian J. Exptl. Biol. Med. Sci.*, **29,** 195 (1951)
40. Babudieri, B., *Rend. ist. super. sanità*, **13,** 472 (1950)
41. Weinman, D., and McAllister, J., *Am. J. Hyg.*, **45,** 102 (1947)
42. Wolff, J. W., and Broom, J. C., *6th Intern. Congr. Microbiol.* (Rome, Italy, September, 1953)
43. Schlossberger, H., Grillo, J., and Scheele, L., *Klin. Wochschr.*, **32,** 1133 (1935)
44. Gispen, R., and Schüffner, W., *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, **144,** 427 (1939)
45. Vaucel, M., *Bull. soc. pathol. exotique*, **30,** 176 (1937)
46. Schlossberger, H., *Z. Hyg. Infektionskrankh.*, **131,** 152 (1950)
47. Bessemans, A., Wittebolle, P., and Niemegeers, L., *Bull. acad. roy. méd. Belg.*, **8,** 442 (1943)
48. Bessemans, A., and Derom, R., *Bull. acad. roy. méd. Belge.*, **12,** 236 (1947)
49. Bessemans, A., and Derom, R., *Rev. belge pathol. et méd. exptl.*, **18,** 391 (1948)
50. Babudieri, B., *Rend. ist. super. sanità*, **10,** 702 (1947)
51. Babudieri, B., and Archetti, I., *Ann. inst. Pasteur*, **75,** 552 (1948)
52. Borg-Petersen, C., *Acta Pathol. Microbiol. Scand.*, **21,** 165 (1944)
52a. Schneider, M. D., *Proc. Soc. Exptl. Biol. Med.*, **82,** 655 (1953)
52b. Schneider, M. D., *Proc. Soc. Exptl. Biol. Med.*, **85,** 32 (1954)
53. Wolff, J. W., *5th Intern. Congr. Microbiol.*, p. 78 (Rio de Janeiro, Brazil, August, 1950)
54. Blindenhöfer, I., *Z. Hyg. Infektionskrankh.*, **129,** 350 (1949)
55. Kritschewsky, J. L., and Tscherikower, R. S., *Z. Immunitätsforsch.*, **46,** 206 (1926)
56. Bessemans, A., and Nélis, P., *Compt. rend. soc. biol.*, **98,** 1234 (1928)
57. Gaethgens, W., *Z. Immunitätsforsch.*, **79,** 428 (1933); **107,** 96 (1950)
58. Randall, R., Wetmore, P. W., and Warner, A. R., Jr., *J. Lab. Clin. Med.*, **34,** 1411 (1949)
59. Broom, J. C., *J. Clin. Pathol.*, **1,** 232 (1948)
60. Borg-Petersen, C., and Fagraeus, A., *Acta Pathol. Microbiol. Scand.*, **26,** 555 (1949)
61. Lübbers, P., *Deut. Arch. klin. Med.*, **198,** 36 (1951)

61a. Schoenherr, K. E., *Z. Immunitätsforsch.*, **110,** 4 (1953)
61b. Stoenner, H. G., *Am. J. Hyg.*, **57,** 316 (1953)
61c. Hoag, W. G., Gochenour, W. S., Jr., and Yager, R. H., *Proc. Soc. Expt. Biol. Med.*, **83,** 490 (1953)
62. Führer, F., *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, **157,** 103 (1951)
63. Wolff, J. W., van Dam, R., and Minkenhof, J. E., *Lancet*, **I,** 1100 (1951)
64. Donatien, H., and Gayot, G., *Arch. inst. Pasteur Algèrie*, **29,** 289 (1951)
65. Zanussi, C., and Squadrini, F., *Riv. ist. sieroterap. ital.*, **26,** 277 (1951)
66. Philipp, E., *Arch. Gynäkol.*, **133,** 573 (1928)
67. Blanc, G., and Delage, B., *Compt. rend. soc. biol.*, **144,** 1632 (1950)
68. Field, H. J., and Sellers, K. C., *Vet. Record*, **63,** 78 (1951)
69. Field, H. J., and Sellers, K. C., *Vet. Record*, **62,** 311 (1950)
70. Anchezar, B. V., Illa, R., and Vivoli, D., *Rev. inst. bacteriol. Malbran*, **14,** 114 (1949)
71. Schlossberger, H., and Langbein, H., *Z. Immunitätsforsch.*, **109,** 366 (1952)
72. Mantovani, G., *Giorn. batteriol. immunol.*, **41,** 374 (1950)
73. Guida, V. O., and Monici, N., *Rev. paulista med. (Sao Paulo)*, **35,** 121 (1949)
74. De Salles Gomes, L., Ribas, J. C., Alvares Corréa, M. O., and Magaldi Jordão, F., *Rev. inst. Adolfo Lutz*, **10,** 111 (1950)
75. Huan-Ying Li, and Davis, D. E., *Am. J. Hyg.*, **56,** 90 (1952)
76. Monlux, W. S., *Cornell Vet.*, **38,** 57 (1948)
77. Reid, E. A. S., and Reed, R. W., *Can. Med. Assoc. J.*, **63,** 479 (1950)
78. Sigurdsson, B., and Sigurdsson, P., *Laeknabladid, Reykjavik*, **35,** 90 (1950)
79. Santos, M., *Rev. brasil. med. Rio de Janiero*, **4,** 424 (1947)
80. Nobrega, P., *Bol. soc. paulista med. vet.*, **8,** 31 (1947)
81. Skrodzki, E., *Med Doświadczalna i Mikrobiol.*, **4,** 291 (1952)
82. Ostertag, H., *Z. Hyg. Infektionskrankh.*, **131,** 482 (1950)
83. Boyer, J., Corre-Hurst, L., Tissier, M., and Sapin-Jaloustre, H., *Presse méd.*, **60,** 445 (1952)
84. Molner, J. G., Meyer, K. F., and Raskin, H. A., *J. Am. Med. Assoc.*, **136,** 814 (1948)
85. Broom, J. C., *Brit. Med. J.*, **II,** 689 (1951)
86. Broom, J. C., and Alston, J. M., *Lancet*, **II,** 96 (1948)
87. Alston, J. M., *Proc. Roy. Soc. Med.*, **42,** 707 (1949)
88. Borg-Petersen, C., *Proc. Roy. Soc. Med.*, **42,** 714 (1949)
88a. Wolff, J. W., and Ruys, A. C., *Documenta Med. Geograph. et Trop.*, **5,** 247 (1953)
89. Goudie, J. G., Weir, I. B. L., and Wilson, T. S., *Glasgow Med. J.*, **233,** 9 (1952)
90. Walch-Sorgdrager, B., and Schüffner, W., *Zentr. Bakteriol. Parasitenk. Abt. I. Orig.*, **141,** 97 (1938)
91. Uhlenhuth, P., Schoenherr, K. E., and Zimmermann, P., *Z. Immunitätsforsch.*, **108,** 1 (1950)
92. Randall, R., and Cooper, H. K., *Science*, **100,** 133 (1944)
93. Bürki, F., *Z. Hyg. Infektionskrankh.*, **135,** 215 (1952)
94. Wirth, D., *Wien. tierärztl. Monatsschr.*, **26,** 353 (1939)
95. Joshua, J. O., *Brit. Vet. J.*, **106,** 321 (1950)
96. Broom, J. C., *Monthly Bull. Ministry Health, Med. Research Council (London)*, **10,** 258 (1951)
97. van Riel, J., Schoenars, F., Brouwers, J., Bienfet, V., and Kaekenbeeck, A., *Rev. belge pathol. et méd. exptl.*, **23,** 38 (1953)

98. Broom, J. C., and Joshua, J. O., *Vet. Record*, **61,** 711 (1949)
99. Steigner, K. F., *Zentr. Bakteriol. Abt. I Orig.*, **156,** 216 (1950)
100. Marcuse, K., and Pohlmann, R., *Zentr. Bakteriol. Abt. I Orig.*, **157,** 490 (1951/52)
101. Wagener, G., *Monatsch. Veterinärmed.*, **6,** 4 (1951)
102. Newman, J. P., *Am. J. Vet. Research*, **11,** 405 (1950)
103. Zaharija, I., *Vet. arhiva (Zagreb)*, **22,** 350 (1952); **23,** 82 (1953)
104. Yamamoto, S., *Japan. J. Vet. Sci.*, **2,** 39 (1940)
105. Gorovitz, H., *Refuah. Vet., Tel-Aviv.*, **6,** 95, 98, 117 (1950)
106. Klarenbeek, A., *Zentr. Bakteriol. I. Orig.* **142,** 83 (1938)
107. Jenny, J., and Kanter, U., *Schweiz. Arch. Tierheilk.*, **88,** 161 (1946)
108. Rimpau, W., and Kalich, J., *Berlin u. Münch. tierärztl. Wochschr.*, **3,** 29 (1948)
109. Minkenhof, J. E., *Lancet*, **II,** 8 (1948)
110. Rosenberg, B. L., *Am. J. Med.*, **11,** 75 (1951)
111. Meyer, K. F., and Brunner, K. T., *Acta tropica*, **7,** 1 (1950)
112. Wirtz, H., *Med. Klin (Münich)*, **23,** 729 (1950)
113. Schermer, J., *Monatsch. Veterinärmed.*, **6,** 1 (1951)
114. Mochtar, A., and Collier, W. A., *Ned. Ind. Blad. Diergeneesk.* (Batavia), **51,** 339 (1939)
115. Mochtar, A., *Japan. J. Vet. Sci.*, **5,** 161 (1943)
116. Wolff, J. W., Loman, S., and Wielenga-Bergman, A. B., *Documenta Neerl. et Indones. Morbis. Trop.*, **3,** 187 (1951)
117. Yamamoto, S., *Office internat. des Epizooties*, No. 193 (1951)
118. van Riel, J., *Ann. soc. belge méd. trop.* **26,** 197 (1946)
119. Schüffner, W., and Bohlander, H., *Zentr. Bakteriol. Abt. I Orig.*, **148,** 263 (1942)
120. Schüffner, W., and Bohlander, H., *Zentr. Bakteriol. Abt. I Orig.*, **149,** 359 (1942)
121. Gsell, O., and Rimpau, W., *Münch. med. Wochschr.*, **91,** 117 (1944)
122. Kathe, J., *Zentr. Bakteriol. Abt. I Orig.*, **155,** 199* (1950)
123. Popp, L., *Z. Hyg. Infektionskrank.*, **131,** 575 (1950)
124. Kathe, J., *Zentr. Bakteriol. Abt. I Orig.*, **152,** 479 (1948)
125. Bernkopf, H., *Harefuah*, **30,** 110 (1946)
126. Bernkopf, H., Olitzki, L., and Stuczynski, L. A., *J. Infectious Diseases*, **80,** 53 (1947)
127. Jacusiel, F., Olitzki, L., and Stuczynski, L., *Harefuah* (Jerusalem), **34,** 84 (1948)
128. Btesh, S., *Trans. Roy. Soc. Trop. Med. Hyg.*, **41,** 419 (1947)
129. Bernkopf, H., Olitzki, L., Stuczynski, L. A., Gotlieb, T., and Halevi, C., *J. Infectious Diseases*, **83,** 232 (1948)
130. Wolff, J. W., and Bohlander, H., *Documenta Med. Geograph. Trop.*, **4,** 257 (1952)
131. Bernkopf, H., and Little, R. B., *Proc. Soc. Exptl. Biol. Med.*, **69,** 503 (1948)
132. Ungar, H., and Bernkopf, H., *Arch. pathol.*, **44,** 59 (1947)
133. Borg-Petersen, C., *Acta Pathol. Microbiol. Scand.*, **27,** 726 (1950)
134. Olejnik, E., and Shneyerson, S., *Trans. Roy. Soc. Trop. Med. Hyg.*, **46,** 165 (1952); *Nature*, **166,** 526 (1950)
135. Gochenour, W. S., Jr., Yager, R. H., and Wetmore, P. W., *Proc. Soc. Exptl. Biol. Med.*, **74,** 199 (1950)
136. Sutherland, A. K., Simmons, G. C., and Kenny, G. C., *Australian Vet. J.*, **25,** 197 (1949)
137. Simmons, G. C., Lawrence, J. J., and Forbes, B. R. V., *Australian Vet. J.*, **28,** 227 (1952)
138. Wikerhauser, T., *Contribution à l'étude des Leptospiroses bovines en Suisse* (Doc-

toral thesis, University of Basel; published by S. Karger, Basel, Switzerland, 1951)
139. Ravaioli, L., and D'Amore, K., *Rend. ist. super. sanità*, **12,** 731 (1949)
139a. Roberts, S. J., York, C. J., and Robinson, J. W., *J. Am. Vet. Med. Assoc.*, **121,** 237 (1952)
139b. Zaharija, I., *Vet. arhiv.* (*Zagreb*), **23,** 318 (1953)
140. Rimpau, W., *Tierärztl. Umschau, No.* 15/16 (1947)
141. Heusser, H., *Schweiz. Arch. Tierheilk.*, **90,** 287 (1948)
142. Heusser, H., Gsell, O., Kanter, U., and Wiesmann, E., *Schweiz. med. Wochschr.*, **78,** 756 (1948)
143. Rossi, P., and Kolochine-Erber, B., *Les Cahiers Méd. Vét.*, **21,** No. 5 (1952)
144. Schlossberger, H., and Schoop, G., *Deut. tierärztl. Wochschr.*, **60,** 393 (1953)
145. Gsell, O., *Bull. Schweiz. Akad. Med. Wiss.*, **1,** 67 (1944)
146. Gochenour, W. S., Jr., Johnston, R. V., Yager, R. H., and Gochenour, W. S., *Am. J. Vet. Research*, **13,** 158 (1952)
147. Wellington, N. A. M., Stevenson, W. J., and Ferris, A. A., *Med. J. Australia*, **2,** 15 (1951)
148. Bordowski, M., *Vo. sanit. Pregl.*, **9,** 90 (1952)
149. van Riel, M. J., *Bull. acad. roy méd. Belg.*, **17,** 368 (1952)
150. Kolochine-Erber, B., and Collombier, M., *Ann. inst. Pasteur*, **79,** 370 (1950)
151. Kirschner, L., Miller, T. F., and Garlick, C. H., *New Zealand Med. J.*, **51,** 98 (1952)
152. Bohl, E. H., and Ferguson, L. C., *J. Am. Vet. Med. Assoc.*, **121,** 421 (1952)
153. Babudieri, B., *Rend. ist. super. sanità*, **12,** 937 (1949)
154. Collier, W. A., *Schweiz. med. Wochschr.*, **78,** 508 (1948)
155. Schmid, G., and Giovanella, R., *Schweiz. Arch. Tierheilk.*, **89,** 1 (1947)
156. Savino, E., and Rennella, E., *Rev. inst. bacteriol. Malbrán*, **14,** 267 (1949)
157. Gsell, O., and Wiesmann, E., *Schweiz. med. Wochschr.*, **21,** 503 (1948)
158. Babudieri, B., *Boll. soc. ital. biol. sper.*, **27,** 104 (1951)
159. Borg-Petersen, C., *Acta Pathol. Microbiol. Scand.*, **21,** 504 (1944)
160. Wolff, J. W., Bohlander, H., and Ruys, A., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **15,** 1 (1949)
160a. Picard, J., *Concours méd.*, **76,** 425 (1954)
161. Lübbers, P., *Z. Hyg. Infektionskrankh.*, **132,** 347 (1951)
162. Babudieri, B., *Rend. ist. super. sanità*, **15,** 704 (1952)
163. Rimpau, W., in *Welt-Seuchen-Atlas*, **I,** 51 (Rodenwalt, E., Ed., Falk-Verlag, Hamburg, Germany, 125 pp., 1952)
164. Borg-Petersen, C., *Proc. Roy. Soc. Med.*, **42,** 714 (1949)
165. Babudieri, B., *Terapia*, **35,** 99 (1950)
166. Babudieri, B., *Rend. ist. super. sanità*, **14,** 133 (1951)
167. Wiesmann, E., *Schweiz. med. Wochschr.*, **76,** 678 (1946)
168. Olitzki, A. L., *Acta Med. Orient.*, **10,** 255 (1951)
169. Collier, W. A., *Acta tropica*, **5,** 135 (1948)
170. Esseveld, H., *J. Trop. Med. Hyg.*, **51,** 171 (1948)
171. Kolochine-Erber, B., Brygoo, E.-R., Cros, R., and Lajudie, P. de, *Ann. inst. Pasteur*, **83,** 1 (1952)
172. Yamamoto, S., *Japan. J. Vet. Sci.*, **10,** 157 (1948)
173. Johnson, D. W., *Med. J. Australia*, **II,** 724 (1950)

173a. Sinnamon, C. N., Pask, V. M., Smith, D. J. W., Brown, H. E., and Tonge, J. I., *Med. J. Australia*, **I,** 887 (1953)
174. Yager, R. H., and Gochenour, W. S., Jr., *Am. J. Trop. Med. Hyg.*, **1,** 457 (1952)
174a. Yager, R. H., and Gochenour, W. S., Jr., *Am. J. Public Health*, **43,** 411 (1953)
174b. Bigham, R. S., *Arch. Internal Med.*, **92,** 587 (1953)
174c. Gochenour, W. S., Jr., and Yager, R. H., *Proc. Am. Vet. Med. Assoc., 89th Meeting*, 178 (Atlantic City, N. J., 1952)
175. Savino, E., and Rennella, E., *Al Atenas* (1946)
176. Savino, E., and Rennella, E., *Leptospira y Leptospirosis en la Republica Argentina* (Buenos Aires, 92 pp., 1944)
177. Savino, E., and Rennella E., *El dia medico*, **18,** 564 (1946)
178. Borg-Petersen, C., and Errebo-Knudsen, E. O., *Nord. Hyg. Tidsskr.*, **5–6,** 109 (1953)
179. Olitzki, L., Stuczynski, L. A., Halevi, C., and Bernkopf, H., *J. Infectious Diseases*, **84,** 15 (1949)
180. Brunner, K. T., and Meyer, K. F., *J. Immunol.*, **64,** 365 (1950)
181. Broom, J. C., *Vet. Record*, **61,** 127 (1949)
182. Donatien, A., and Gayot, G., *Arch. inst. Pasteur Algérie*, **29,** 280 (1951)
183. Wylie, I. A. H., and Vincent, E., *J. Pathol. Bact.*, **59,** 247 (1947)
184. Borg-Petersen, C., and Rotwitt Schmidt, M., *Acta Pathol. Microbiol. Scand.*, **22,** 462 (1945)
185. Heilman, F. R., *Proc. Staff Meetings Mayo Clinic*, **23,** 569 (1948)
186. Brunner, K. T., and Meyer, K. F., *Am. J. Vet. Research*, **11,** 89 (1950)
187. Uhlenhuth, P., and Schoenherr, K. E., *Med. Klin. Munich*, **46,** 859 (1951); *Z. Immunitätsforsch.*, **108,** 289 (1951)
188. Schlipköter, H. W., *Münch. med. Wochschr.*, **93,** 2300 (1951)
189. Fühner, F., *Deut. Z. Verdauungs- u. Stoffwechselkrankh.*, **12,** 82 (1952)
190. Schlipköter, H. W., and Gram, H., *Z. Immunitätsforsch.*, **109,** 215 (1952)
191. Gädeke, R., and Schoenherr, K. E., *Z. Immunitätsforsch.*, **109,** 488 (1952)

LIFE HISTORY OF MALARIA PARASITES[1]

By P. C. C. Garnham

Department of Parasitology, London School of Hygiene and Tropical Medicine, London, England

Seven years ago, Huff (50) reviewed the existing state of knowledge of the life history of malaria parasites, confining his survey largely to the tissue stages of the organisms. At that time the evidence for the existence of these stages in mammalian malaria was chiefly inferential; Huff nevertheless concluded his review by stating that (*a*) the evidence was so good that it warranted the adoption of the idea as a working hypothesis and (*b*) the tissue stages in mammalian malaria might not only differ from the avian forms but also from each other. The present review will show the accuracy of his prophecy.

Since 1947, several reviews of the subject have appeared, including the Croonian lectures by Fairley (22) and a summary by Garnham (29); of particular interest are papers by Raffaele (83), Sergent (96), and Mudrow (73). The chief advance in knowledge has been the discovery of tissue stages of many mammalian parasites including the malaria parasites of man. However, important details of the life history in other respects have also been worked out recently, including the physiology of the organism as revealed by new cultural methods, studies on the nucleus, gametocytogenesis, infection in the mosquito, the relapse phenomenon, etc. Full information on these subjects is given in the new edition of Boyd's "Malariology" (4) and in Russell (91). Several new parasites have been described, the most important of which is probably the rodent parasite, *Plasmodium berghei*. It is extraordinary in a subject as extensively studied as malaria that fundamental aspects are still being disclosed. The purpose of this review is to try to give a survey of the more significant discoveries in relation to the tissue stages, together with a résumé of new species of parasites.

The definition of the genus has altered since Marchiafava & Celli (68) gave the original description. For the purpose of this paper, malaria parasites are considered to be pigmented parasites of erythrocytes, including genera with and without schizogony in the erythrocyte. All parasites of the suborder Haemosporidiidea are therefore included with the exception of *Leucocytozoon*. This is necessary because of the importance to the subject of certain mammalian parasites, such as *Hepatocystis* (=*Plasmodium*) *kochi*, and because some authors [e.g., Hoogstraal *et al.* (49)] refuse at present to accept new generic names, retaining them still in the genus *Plasmodium*. Problems of classification are complex and have received attention in recent years from Christophers (10), Garnham (36), and others.

[1] The survey of literature pertaining to this review was completed in January, 1954.

Tissue Stages of Mammalian Malaria Parasites

Before 1947, there was no clear-cut evidence of the existence of exo-erythrocytic schizogony in mammalian malaria, though present day research indicates that the forms described by Schingarew (92) in bats, Levaditi & Schoen (62) and Schwetz (95) in monkeys, and Manwell (66) in flying foxes were of this type. The nature of the bodies found in human bone-marrow smears by Raffaele (82) and others was discussed by Huff (50) in his review; they are referred to again below.

Parasites with gametocytes only in the blood.—In 1947, Mer & Goldblum (71) briefly described the exo-erythrocytic cycle of a parasite of Palestine bats—probably "*P.*" *murinum*. They demonstrated that the infection is transmitted by nycteribiid flies and that early exo-erythrocytic development takes place in fixed reticulo-endothelial cells of the bone-marrow, spleen, lungs, and kidney. Soon afterwards, the endothelium lining the liver sinusoids becomes the seat of invasion. The tissue forms do not persist for long, but they reappear before a relapse. Goldblum has continued his researches on this parasite, but the results are available at present only in manuscript form in Jerusalem.

Shortly afterwards, Garnham (28, 30) described the details of exo-erythrocytic schizogony of *H. kochi* in the lower monkeys of East Africa. The smallest forms were found by the late Dr. C. M. Wenyon in the author's sections; they were round bodies with peripheral nuclei contained in parenchyma cells of the liver. With the growth of the parasite, the host cell expands and its nucleus divides several times, becoming much enlarged, although not to the same extent as the host cell nucleus of *Leucocytozoon*. The nuclei of the parasite lose their peripheral distribution, and when the parasite is at this stage, measuring about 60 μ or rather more, it bears a close resemblance to some of the human forms. Later, the surface becomes much convoluted, vacuoles form in the cytoplasm, growth continues, and finally the vacuoles condense into a large central sac filled with fluid, lined by cytoplasm with closely packed nuclei. This body measures about 2 mm. and is visible as a transparent cyst (the merocyst) on the surface of the liver. Condensation of cytoplasm takes place around each nucleus, and the merozoites escape, probably over a considerable period, into the sinusoids to invade erythrocytes and become gametocytes. The structure of the merocyst differs slightly in baboons and other monkeys kept for a long time in captivity; Garnham & Pick (40) showed that the forms could then reach 3 mm. in diameter and possessed a curious digitiform irregular border. Haddow (43) found merocysts of both sizes in 36 per cent of red tail monkeys in Uganda, but rarely in *Papio*, *Cercocebus*, and *Colobus*. In 1949, Ray (84) described merocysts, much resembling the latter type, in the liver and spleen of Himalayan flying squirrels infected with an unnamed malaria parasite. The illustrations accompanying his paper do not include forms in the spleen, and the presence of exo-erythrocytic schizogony in this organ has not been confirmed.

Field & Edeson (23) next found merocysts in the livers of true squirrels in Malayan forests; the blood of the animals showed heavy infections of gametocytes of *Hepatocystis vassali malayensis*. The mature tissue forms were less than half the size of the merocysts of *H. kochi* but showed a similar structure and were accompanied by a suspected early stage—solid, and measuring 60 μ by 35 μ.

Certain species of fruit bats or flying foxes of tropical Africa are infected with *H. epomophori*, and exo-erythrocytic schizogony of the parasite has been found by Rodhain (90) and Garnham (32, 35). It occurs in the liver in the form of merocysts with digitiform borders, evolving from solid schizonts with extremely dense cytoplasm.

Lastly in this group comes another parasite of African bats, which has such anomalous features that it had to be placed in a new genus: *Nycteria medusiformis* [Garnham & Heisch (39)]. Gametocytes alone are found in the blood, and asexual development is apparently confined to parenchyma cells of the liver, wherein grow solid schizonts closely resembling the pre-erythrocytic forms of *P. falciparum*. This similarity is however confined only to the size and bizarre contour; the nuclear structure is quite different and may, in fact, represent suspended telophase.

The discovery of stages of exo-erythrocytic schizogony in species of *Hepatocystis* was largely the result of examining material with the naked eye or the low power of the microscope; the failure of earlier workers to demonstrate them was probably a consequence of the natural practice of malariologists of using high magnifications in studying the parasite. By doing so they inevitably missed the sparsely distributed merocyst. Work on these parasites showed that development begins in the parenchyma cell of the liver, and later work on the tissue forms of true *Plasmodium* confirmed that these also grow in the same situation.

Further study of *Hepatocystis* and *Nycteria* is handicapped at present by ignorance of the nature of the vector. Details of the pre-erythrocytic cycle are unknown, because observations have been confined to animals infected in nature and the length and characters of these infections can never be exactly determined. Repeated attempts to find the vectors have met with failure [see, amongst others, Bearup & Lawrence (2), McGhee (69), Rodhain (85), Mulligan & Sommerville (75), Garnham (34)]; it seems probable that the vector is some arthropod other than a mosquito.

Parasites with schizonts and gametocytes in the blood.—This group of parasites includes the malaria parasites of man; it is apparently linked to the former group by an East Indian species, *P. knowlesi*, which exhibits exo-erythrocytic schizonts of the *Hepatocystis* type. Although little is yet known about its tissue stages, *P. knowlesi* may usefully therefore be discussed first. The parasite is fairly common in kra monkeys in Malaya and the East Indies, and in 1953 Edeson (20) reported the presence of presumed exo-erythrocytic schizonts in five monkeys showing *P. knowlesi* in the blood stream. They were in the form of merocysts up to 1.5 mm. or more in diameter, often possessing

the finger-like processes or the "daughter cyst" formation described by Ray (84) in the parasite of *Petaurista*. Immature solid forms about 100 μ across were also seen. At the same time, Hawking & Mellanby (46) stated that they had been able to transmit *P. knowlesi* [a new strain identified by Jaswant Singh *et al.* (58)] by *Anopheles stephensi*, maintained on a diet containing *p*-aminobenzoic acid. Light infections of the salivary glands were obtained, and these glands were inoculated intravenously into a rhesus monkey, which developed parasitaemia eight days later. They thus demonstrated that pre-erythrocytic schizonts of *P. knowlesi* could reach maturity in eight days or less. It seems most improbable that Edeson's merocysts could develop in such a short space of time, and it is more likely that they represent later exo-erythrocytic than pre-erythrocytic forms. Shortt & Bray (97) have found "giant" schizonts of *P. cynomolgi* late in the infection, and Edeson's merocysts may be of an analogous type.

Pre-erythrocytic schizogony of *P. cynomolgi* was first demonstrated by Shortt, Garnham & Malamos (105) in 1948. This species closely resembles *P. vivax*, and work on its tissue stages has thrown much light on problems of the life cycle of the human parasite. The tissue stage of *P. vivax* itself was discovered by Shortt *et al.* (104) later in 1948, of *P. falciparum* by Shortt *et al.* (100) in 1949, and of *P. ovale* by Garnham *et al.* (37) in 1954. The pre-erythrocytic stages of the human quartan malaria parasite remain unknown, but work done by Garnham (31) on *P. inui* (an almost identical parasite of lower monkeys) indicates what they are likely to be.

The successful outcome of this research was primarily dependent on the realization of the magnitude of the organ or organs likely to be the seat of pre-erythrocytic schizogony in comparison to the usually tiny infecting dose of sporozoites. Under natural conditions the hope of tracing developmental stages of the sporozoite would be as remote as finding a needle in a haystack. Therefore, the use of very large numbers of heavily infected mosquitoes was regarded as essential, and, in order to provide the sporozoite with as easy a passage to its developmental site as possible, intravenous inoculations of salivary glands was the usual method employed. Examination of sections under low magnification enabled a comparatively large amount of material to be searched. Finally, the use of the Shortt-Cooper (99) Giemsa colophonium method produced the Romanowsky colour contrasts in tissue parasites and gave ideal pictures of the different stages.

In the original research both on *H. kochi* and *P. cynomolgi*, all organs and tissues of the infected animals were searched for the presence of parasites; no tissue forms were found anywhere except in the liver, where they were seen to develop solely in the parenchyma.

P. cynomolgi: Recent descriptions of the erythrocytic life cycle of this simian parasite have been given by Wolfson & Winter (116) and by the Taliaferros (106), and the curious behaviour of the parasite in splenectomised animals has been analysed by Schmidt (93). The tissue stages have been studied by Shortt & Garnham (101, 102), Hawking *et al.* (47), Bray & Garnham (7),

and by Shortt, Bray & Cooper (98). Pre-erythrocytic schizogony of *P. cynomolgi* is known from two days onwards. At that time the parasite is a round or oval body, 2.5 μ long with an undivided nucleus. By the fourth day the schizont is a compact body 11 μ in diameter with about 24 nuclei. With further growth the nucleus of the host cell is pushed aside, and one or more vacuoles appear in the cytoplasm of the parasite. At maturity on the eighth day the schizont measures about 38 μ and contains about 10,000 merozoites. A few parasites continue growing for a few days longer and, exceptionally, by the fifteenth day may reach the gigantic diameter of 108 μ [Shortt *et al.* (98)]. Most of the merozoites derived from these schizonts enter erythrocytes, but a few return to the parenchyma, where they initiate the exo-erythrocytic cycle. The latter persists for a long period, and such exo-erythrocytic forms were demonstrated by Shortt & Garnham (103) 3½ months after the original infection. It is highly probable that the latter forms are responsible for relapses. The morphology of pre- and exo-erythrocytic schizonts is practically the same.

P. vivax: The pre-erythrocytic stage of this parasite is known only from the experiment on a human volunteer of Shortt *et al.* (104). Forms approaching maturity were found; they were much like the schizonts of *P. cynomolgi*, being of regular contour and containing vacuoles, but were slightly larger (42 μ) and probably contained more merozoites (about 12,000). Indirect evidence regarding long term relapses [Fairley (22)] and latency strongly suggests that exo-erythrocytic schizonts of *P. vivax* persist in the liver.

P. falciparum: Shortt *et al.* (100) and Jeffery *et al.* (59) have demonstrated the pre-erythrodytic cycle of this parasite. The youngest forms measured about 15 μ in diameter and probably represented three days' growth. Development is very rapid, and the parasite pushes out in any direction where pressure from the surrounding tissue is least; this results in the formation of lobes and protuberances on the surface of the schizont. No vacuoles form. Early on the sixth day, the parasite is mature, measuring approximately 60 μ and containing about 40,000 merozoites. Details of the subsequent life history of this parasite (e.g., absence of true relapses) suggests that no exo-erythrocytic schizogony follows.

P. inui: It is lucky that a simian form of quartan malaria exists, because for various reasons details of the tissue stages of the human parasite would be excessively difficult to elicit. *P. inui* however closely resembles *P. malariae* in its 72 hr. blood cycle, in the duration of sporogony, and in general morphology; its pre-erythrocytic cycle was determined by Garnham (31), and this probably as closely parallels that of the human parasite as the cycle of *P. cynomolgi* does that of *P. vivax*. The earliest form in the liver was found seven days after inoculation of sporozoites and is a compact spherical body, with a clear-cut border, only 5.5 μ in diameter. Cytoplasm is tenuous, growth is slow, and maturity is not reached until the eleventh day. Shortly before maturity, the parasite undergoes a remarkable transformation into "cytomeres", and there is sometimes a palisade arrangement of nuclei reminiscent both of

the so-called sporoblasts in oöcysts and of certain exo-erythrocytic stages in avian malaria. The mature schizont is a spherical body measuring about 24 μ in diameter and containing about 2000 merozoites. Long term relapses [Nicolajev (78)] are common in quartan malaria, and it is probable that exo-erythrocytic parasites remain in the liver for years.

P. ovale: Knowledge of the tissue stage of this parasite should provide useful confirmation of its specific status, and recent work by Garnham *et al.* (37) with human volunteers has, in fact, elicited its nature. Full details of the pre-erythrocytic stages are still unknown, but the following observations suffice to illustrate the differences between *P. ovale* and the other parasites of human malaria: a prepatent period of nine days, the size (42 μ) and irregularity of the mature schizont, the excessively large size of the nuclei, and the suggestion of division into "cytomeres."

Table I gives the differential characters of the tissue forms of the parasites mentioned above.

TABLE I

DIFFERENTIAL CHARACTERS OF THE TISSUE PHASE OF MAMMALIAN MALARIA PARASITES

Species	Length of Pre-erythrocytic Cycle	Size of Mature Schizont	Approximate Number of Merozoites	Special Features	Existence of Exo-erythrocytic Cycle
P. cynomolgi	8 days	38μ	under 10,000	vacuoles	Yes
P. vivax	8 days	42μ	over 10,000	vacuoles	Probable
P. falciparum	6 days	60μ	40,000	irregular contour	Improbable
P. inui	11 days	24μ	2000	"Cytomeres"	Probable
P. ovale	9 days	45μ	?10,000	Large nuclei	Probable

A few workers have criticised the validity of the above claims that pre- and exo-erythrocytic stages have been found in mammalian malaria. Huff (51, 55) was unwilling to accept them outright because (*a*) there were no controls, (*b*) there was not a complete series of stages from the sporozoite to the mature schizont, and (*c*) the staining reaction of the so-called parasites was unlike that of the exo-erythrocytic forms he had himself so successfully demonstrated in avian malaria. Since this criticism was made the results of experiments in which controls were used have been published [Shortt, Bray & Cooper (98)], and many gaps between the stages have now been filled in. In addition, experimental inoculation of sporozoites into monkeys at specific intervals has been shown to be followed by the appearance of pre-erythrocytic schizonts of predictable sizes in the liver, and Bray (5) has indicated that the number of such schizonts is roughly proportional to the number of sporozoites inoculated. Histochemical reactions provide a neat confirmation of the

nature of the parasites while in process of growth. Bray & Garnham (6) showed that desoxyribonucleic acid was absent from the nuclei of the younger forms of *P. inui* but was present as the parasite became mature, when the nuclei gave a strongly positive Feulgen reaction. These results are similar to those obtained by Lewert (64) working on exo-erythrocytic forms of *P. gallinaceum*.

Coulston & Robinson (14) demonstrated the similarity between platelet accumulations in sections of liver and maturing pre-erythrocytic schizonts of *P. cynomolgi*. It is certainly true that blood platelets may be mistaken sometimes under low magnification for this form of the parasite, but under higher powers and with adequate staining the artefact may easily be distinguished.

Another criticism concerns the location of the developing parasite. The youngest pre-erythrocytic schizonts have been found in parenchyma cells two days after intravenous inoculation of sporozoites of *P. cynomolgi* by Shortt *et al.* (98); these authors are convinced that the sporozoite enters such cells from the first and that only one (cryptozoic) cycle takes place during the prepatent period. Mudrow (73) originally suggested that a preliminary cryptozoic cycle in the Kupffer's cells of the liver was more likely on theoretical grounds, and she thought this would be followed by the second (metacryptozoic) cycle in the parenchyma. Some support for her view was provided by the work of Coulston (12), who actually demonstrated sporozoites, and parasites evolving from them, in the Kupffer cells 44 hr. after intravenous inoculation. These forms closely resembled similar stages in avian malaria. Nevertheless, this phenomenon could be the result of phagocytosis. The first 48 hours thus still remain a mystery, and it is impossible at present to be certain what happens during them. Raffaele (83) considers that the pre-erythrocytic forms grow in the Kupffer cells of the liver because of the irregularity of their contour; if the parasite grew in a hepatic cell then its shape should be more round or oval. In most parasites this is the case, and the demonstration of young forms of *P. inui* and *P. cynomolgi* clearly inside parenchyma cells puts the matter beyond doubt. There is nothing unusual or unexpected about the development of a coccidian parasite in the parenchyma cells of the liver; there are in fact three reasons for thinking that this should be the natural site: (*a*) phylogenetically these organisms probably derive from coccidia developing in the intestinal epithelium, which has the same endodermal origin as the parenchyma cells of the liver; (*b*) the unique richness of the parenchyma in nucleo-proteins and amino acids makes it particularly suitable for parasites undergoing rapid nuclear multiplication; and (*c*) the parenchyma cell is the well-known location of other allied parasites, such as *Hepatozoon* [see Miller (72), and Brumpt (8)].

Indirect evidence that growth of sporozoites of mammalian malaria does not take place in reticulo-endothelial cells is provided by the negative results of tissue culture experiments of Dubin *et al.* (19) and others; avian parasites, whose normal location is the reticulo-endothelium and other parts of the mesoderm, on the contrary grow easily in tissue culture.

Huff (50) discussed the claims of various workers to find exo-erythrocytic

schizogony in various organs, particularly in the bone-marrow. He pointed out that there was little consistency amongst the descriptions and that many of them represented artefacts. Since that time, additional findings of a similar nature have been reported by Oberlé (79), da Fonseca *et al.* (25), Garcia (27), Inoki (57), Blanc & Languillon (3), Décourt (15), and Gallais *et al.* (26). The discovery of clear-cut tissue cycles in the liver in most forms of mammalian malaria now clearly indicates that this is the usual form of development of the parasites. Ascoli & d'Alessandro (1) have pointed out, however, that there are certain aspects of relapses in malaria which cannot be explained on this basis, particularly the suddenness of attack after provocation. They think that such relapses are attributable to contractions of the spleen squeezing hidden parasites into the peripheral circulation. Ascoli himself considers that these relapse forms are endo-erythrocytic, but other workers [e.g., Gallais *et al.* (26)] think that they are exo-erythrocytic. It appears to the reviewer possible though very unlikely that the forms in the bone-marrow or elsewhere represent a special tissue cycle; a much more probable explanation is that they either are products of phagocytosis or are accidentally exo-erythrocytic, just as *P. berghei* sometimes develops free in the plasma or in erythroblasts and other cells containing no haemoglobin.

Exo-erythrocytic schizogony in avian and saurian malaria parasites.—The major discoveries in this field were made before 1947 and were described by Huff (50). Possibly the greatest advance since that date was the growth in tissue culture of several avian plasmodia. Hawking (44) succeeded first with *P. gallinaceum*, using pieces of spleen and other organs taken from chicks inoculated 8 to 9 days previously with sporozoites; later Dubin *et al.* (18) inoculated sporozoites directly into cultures of macrophages and obtained vigorous growth. In addition to *P. gallinaceum*, tissue culture of the following species has been successful: *P. relictum* [Hawking (45)], *P. lophurae* [Tonkin & Hawking (108)], and *P. cathemerium* [Hegner & Wolfson (48)]. Zuckermann (118) and others have succeeded in growing *P. gallinaceum* in the chick embryo, and some of her recent work suggests that in its exo-erythrocytic life history this parasite is not confined to fixed reticulo-endothelium but may take on *elongatum* features.

Huff (52) has compared pre- and exo-erythrocytic schizogony of several species of avian malaria in different hosts. In completely unsuitable birds, no development of the sporozoite takes place; in birds with some degree of natural immunity, the sporozoite proceeds to the cryptozoic stage but no further; with less immunity, the damaged pre-erythrocytic stages may be completed, but the resultant parasitaemia is slight; finally, in completely susceptible birds, the tissue and blood stages run their full course. In the various host-parasite combinations, pre-erythrocytic stages were found in 62 per cent, and blood stages in only 29 per cent.

Dimorphism of tissue stages (into macro- and micro-merozoites) is still under discussion. In avian malaria the phenomenon had been accepted following the observations of Mudrow & Reichenow (74) and many others, who thought that macro-merozoites were destined for further invasion of tissue

cells and micro-merozoites for blood cells. Recently Huff (53) has shown that a complete gradation of sizes is present, and he suggests that no true differentiation exists, the size being dependent upon the type of cell invaded.

Downs (16) inoculated a single erythrocytic form of *P. gallinaceum* into a chick, which became infected and gave rise, in subsequent passages, to exo-erythrocytic stages of the parasite. This fundamental observation confirms the earlier work of Coulston & Manwell (13) on inoculations of single parasites of *P. circumflexum.*

Recent work on the exo-erythrocytic stages of avian and saurian species is considered briefly below.

P. gallinaceum: Haas *et al.* (42) gave an interesting description of the response of exo-erythrocytic forms to changes in the life cycle of the parasite and indicated that three patterns could be distinguished: (*a*) after blood inoculation, (*b*) after mosquito transmission, and (c) after inoculation of various suspensions of organs, etc. Artificial changes in the life cycle can produce strange results: if a bird is inoculated with exo-erythrocytic forms grown in tissue culture, the resultant infection will sometimes be entirely exo-erythrocytic in character [Lewert (63)]; if blood from chicks of an alternate mosquito-blood infection series is inoculated into embryos, the erythrocytic parasites will all exhibit large vacuoles, a peculiarity found to be transmissible by subsequent mosquito passage [Wilcox *et al.* (115)]. Although they do not specifically concern the tissue phase, the recent experiments of Weathersby (114) illustrate most extraordinary modifications in the life history of *P. gallinaceum:* Inoculation of blood containing gametocytes into the haemocoele of suitable mosquitoes was followed by the appearance of oöcysts in nearly all parts of the body of the insect (even in the palps) and finally by invasion by sporozoites of the salivary glands. The mid-gut played no part in the artificial cycle. Corradetti & Cavaceppi (11) determined the length and course of the exo-erythrocytic cycle by repeated biopsies of brain; they showed that this cycle persisted for a maximum of 25 days and then demonstrated the resistance (or acquired immunity) of the capillary endothelium to subsequent infection, although the l[illegible]ntinued to exhibit a low grade parasitaemia.

P. lophurae: It was [illegible]ginally thought that exo-erythrocytic stages of this parasite occurred only after mosquito transmission, but Manresa (65) has now shown that blood inoculation of the parasite into turkeys will give rise to tissue forms. By ingenious methods, McGhee (70) has succeeded in establishing this avian species in baby mice; its morphology however was changed: gametocytes were no longer present, and phanerozoites were absent.

P. cathemerium: Mudrow & Reichenow (74) give a detailed account of the pre- and exo-erythrocytic stages of this parasite in canaries.

P. juxtanucleare: Mosquito transmission of this parasite of the domestic fowl has not been effected, but exo-erythrocytic stages have been briefly described by Paraense (80) after blood passage. The schizonts were apparently in macrophage or endothelial cells of the spleen and other organs and contained up to 80 merozoites.

P. fallax: This parasite was originally found by Schwetz (94) in Congo

owls; it was rediscovered in the Southern Sudan in guinea fowls and has since been extensively studied by Huff *et al.* (56). Pre-erythrocytic schizogony was discovered in various wandering cells in the skin and later in the organs; it is now said [Huff (55)] that exo-erythrocytic schizonts can be produced after blood inoculation.

P. hexamerium: This was the first species of the "small avian" group in which tissue stages were found—viz. by Manwell in 1951 (67) in naturally infected warblers. The exo-erythrocytic schizonts resembled those of *P. gallinaceum.*

P. vaughani: This small avian parasite was shown by Laird (61) to possess exo-erythrocytic schizonts of the *elongatum* type. They were found in erythroblasts, lymphocytes and macrophages.

P. huffi: Exo-erythrocytic schizonts of this parasite have been shown by Muniz *et al.* (77) and by Huff (54) to belong to the *elongatum* type; they were found in macrophages and granulocytes, but principally in lymphocytes in the bone-marrow and spleen.

Saurian malaria parasites: Thompson & Huff (107) described the tissue cycle of *P. mexicanum* and showed that it possesses both *gallinaceum* and *elongatum* features. The invertebrate host of this and of all other saurian plasmodia remains unknown in spite of the intensive study on *P. floridense* by Goodwin (41), who elucidated many other details of its life history. Exo-erythrocytic schizonts of *P. pitmani* of African skinks were found by Garnham (33) in reticuloendothelial cells of the spleen and heart, and of *P. agamae* by Garnham & Duke (38) in monocytes of the blood. Laird (60) found small exo-erythrocytic forms (with up to 20 nuclei) in thrombocytes and lymphocytes in heart blood of New Zealand skinks infected with *P. lygosomae* (*?= lacertiliae*). Until the arthropod vector is established and details of pre-erythrocytic schizogony are obtained, the exact nature of the tissue phase in all these parasites of lizards will remain unknown.

New Species of Malaria Parasites

Surveys of the blood of wild animals have continued to reveal new species of *Plasmodium* and to provide confirmation of old and little-known ones. The most important of the former group is undoubtedly *P. berghei* and of the latter *P. fallax.* Perhaps in the latter category should be included also the recent work by Rodhain (87, 88) establishing the specificity of the malaria parasites of chimpanzees: he has shown by sub-inoculation of blood into man and ape that *P. reichenowi* and *P. schwetzi* are distinct species, but that *P. rodhaini* is identical with *P. malariae.* The chimpanzee is thus a potential animal reservoir of quartan malaria. These discoveries have shed new light on the life history of malaria parasites but cannot be considered in detail here; the literature on *P. berghei* alone is now enormous [see Draper (17)], and it is only possible to give a very brief description in this review.

P. berghei: This species was discovered by Vincke & Lips (111) in 1948 after a series of brilliant researches, the stages of which may be summarised

as follows: (*a*) discovery of *Anopheles dureni* in gallery forest of the Belgian Congo [Evans (21)]; (*b*) finding of sporozoites in the salivary glands of many wild adults of this species [Vincke *et al.* (112)]. (*c*) demonstration by precipitin tests of the stomach contents of the mosquitoes that the contained blood was not that of any of the well-known animals or of man; and (*d*) eventual recovery of a *Plasmodium* in forest rats of the genus *Thamnomys*. *P. berghei* will flourish in a large number of different rodents, but so far no really suitable species of laboratory colonized *Anopheles* has been found to maintain it. Yoeli & Wall (117) just managed to achieve transmission with *A. maculipennis atroparvus;* Perez-Reyes (81) is apparently having greater success with *A. aztecus*. Until the mosquito cycle is established with ease, it is unlikely that the full life cycle of the parasite will become known. A highly significant finding is the recent work of Vincke & Peeters (113) which demonstrated that the pre-erythrocytic cycle is as short as 48 hr. This suggests that *P. berghei* is probably more closely allied to the avian than to the other mammalian parasites and that the tissue stages will be found in the reticuloendothelium rather than in the parenchyma cells of the liver. The so-called exo-erythrocytic forms of the parasite described by Van den Berghe *et al.* (109) after blood inoculation probably do not represent a fundamental stage in the cycle.

P. vinckei was described by Rodhain (89) and is closely allied to *P. berghei*. *P. hylobati* was found in a gibbon from Java by Rodhain (86) and resembles *P. inui* of lower monkeys. *P. roussetti* was found in fruit bats inhabiting caves in the Eastern Congo by van Riel *et al.* (110) and is of interest because probably it is the sole representative of the genus *Plasmodium sensu strictu* in bats. The infection is transmitted congenitally to the foetus. *P. girardi* was found by Bück *et al.* (9) in lemurs in Madagascar. It is of the quartan type. *P. simium* was described by da Fonseca (24) from howler monkeys in São Paulo, Brazil. The parasite is the South American representative in lower monkeys of the *vivax* series, the African one being *P. gonderi* and the Asian, *P. cynomolgi*. *P. huffi* was found by Muniz *et al.* (77) in toucans, and so far this bird has proved to be the only suitable host. Its exo-erythrocytic stages are described above. *P. pinottii*, an avian parasite recently isolated by Muniz (76) in Brazil, is likely to prove of considerable experimental interest because of the wide range of birds which have been found susceptible.

LITERATURE CITED

1. Ascoli, M., and d'Alessandro, G., *Acta Tropica*, **7**, 367 (1950)
2. Bearup, A. J., and Lawrence, J. J., *Proc. Linnean Soc. N. S. Wales*, **71**, 197 (1947)
3. Blanc, F., and Languillon, J., *Med. trop.*, **9**, 336 (1949)
4. Boyd, M. F., Ed., *Malariology* (W. B. Saunders Co., Philadelphia, Pa. & London, England, 1643 pp., 1949)
5. Bray, R. S., *The Exo-erythrocytic Cycle of Plasmodium* (Doctoral thesis, unpublished, University of London, London, England, 1954)
6. Bray, R. S., and Garnham, P. C. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, 263 (1953)

7. Bray, R. S., and Garnham, P. C. C., *Brit. Med. J.*, **I**, 1200 (1953)
8. Brumpt, E., *Ann. parasitol. humaine et comparée*, **21**, 1 (1946)
9. Bück, G., Coudurier, J., and Quesnel, J. J., *Arch. inst. Pasteur Algérie*, **30**, 240 (1952)
10. Christophers, S. R., *Rev. inst. salubridad y enfermedad. trop.* (*Mex*), **6**, 213 (1945)
11. Corradetti, A., and Cavaceppi, L., *Am. J. Trop. Med. Hyg.*, **1**, 761 (1952)
12. Coulston, F., *Proc. Soc. Exptl. Biol. Med.*, **70**, 360 (1949)
13. Coulston, F., and Manwell, R. D., *Am. J. Hyg.*, **34**, 119 (1941)
14. Coulston, F., and Robinson, F. O., *J. Parasitol.*, **36**, 28 (1950)
15. Décourt, P., *Bull. soc. pathol. exotique*, **42**, 452 (1949)
16. Downs, W. G., *Am. J. Hyg.*, **46**, 41 (1947)
17. Draper, C. C., *Plasmodium berghei Vincke and Lips, 1948. A bibliography of published papers up to April, 1953.* (Unpublished)
18. Dubin, I. N., Laird, R. L., and Drinnon, V. P., *J. Nat. Mal. Soc.*, **8**, 175 (1949)
19. Dubin, I. N., Laird, R. L., and Drinnon, V. P., *J. Natl. Malaria Soc.*, **9**, 119 (1950)
20. Edeson, J. F. B., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, 399 (1953)
21. Evans, A. M., *Mosquitoes of the Ethiopian Region*, 3 vols., **II**, *Anophelini adults and early stages* (British Museum Natural History, London, Engl., 404 pp., 1938)
22. Fairley, N. H., *Brit. Med. J.*, **II**, 825 (1949)
23. Field, J. W., and Edeson, J. F. B., *Bull. Inst. Med. Research Federation Malaya* (**II**, 1949, **1**, 1950)
24. da Fonseca, F., *Mem. inst. Oswaldo Cruz*, **49**, 543 (1951)
25. da Fonseca, F., Cambournac, F. J. C., Pinto, M. R., Pereira, J. M., and Cunha, A., *Parasitology*, **37**, 113 (1946)
26. Gallais, P., Cros, R., and Capponi, M., *Med. Trop.*, **9**, 307 (1949)
27. Garcia, E. Y., *Clin. Excerpts* (*Manila*), **2**, 22 (1950)
28. Garnham, P. C. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, 719 (1947)
29. Garnham, P. C. C., *Trop. Diseases Bull.*, **45**, 831 (1948)
30. Garnham, P. C. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, 601 (1948)
31. Garnham, P. C. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **45**, 45 (1950)
32. Garnham, P. C. C., *Nature*, **166**, 155 (1950)
33. Garnham, P. C. C., *Parasitology*, **40**, 328 (1950)
34. Garnham, P. C. C., *Exptl. Parasitol.*, **1**, 94 (1951)
35. Garnham, P. C. C., *Riv. malariol.*, **32** (In press)
36. Garnham, P. C. C., *Proc. 5th Internat. Congr. Trop. Med. Malaria* (Istanbul, Turkey, August–September, 1953) (In press)
37. Garnham, P. C. C., Bray, R. S., Cooper, W., Lainson, R., Awad, F. I., and Williamson, J., *Brit. Med. J.*, **I**, 257 (1954)
38. Garnham, P. C. C., and Duke, B. O. L., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, 7 (1953)
39. Garnham, P. C. C., and Heisch, R. B., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, 357 (1953)
40. Garnham, P. C. C., and Pick, F., *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, 535 (1952)
41. Goodwin, M. H., Jr., *J. Natl. Malaria Soc.*, **10**, 57 (1951)
42. Haas, V. H., Wilcox, A., Laird, R. L., Ewing, F. M., and Coleman, N., *J. Parasitol.* **34**, 306 (1948)
43. Haddow, A. J., *Proc. Zool. Soc.* (*London*), **122**, 297 (1952)

44. Hawking, F., *Trans. Roy. Soc. Trop. Med. Hyg.*, **39,** 245 (1945)
45. Hawking, F., *Trans. Roy. Soc. Trop. Med. Hyg.*, **40,** 183 (1946)
46. Hawking, F., and Mellanby, H., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47,** 438 (1950)
47. Hawking, F., Perry, W.L.M., and Thurston, J. P., *Lancet*, **I,** 783 (May 22, 1948)
48. Hegner, R., and Wolfson, F., *Am. J. Hyg.*, **29,** 83 (1939)
49. Hoogstraal, H., Huff, C. G., and Lawless, D. K., *J. Natl. Malaria Soc.*, **9,** 293 (1950)
50. Huff, C. G., *Ann. Rev. Microbiol.*, **I,** p. 47 (1947)
51. Huff, C. G., *Trop. Med. Hyg. News*, **7,** 3, 22 (1950)
52. Huff, C. G., *J. Infectious Diseases*, **88,** 17 (1951)
53. Huff, C. G., *Exptl. Parasitol.*, **1,** 392 (1952)
54. Huff, C. G., *Am. J. Trop. Med. Hyg.*, **2,** 620 (1953)
55. Huff, C. G., Discussion following report on parasitology, premunition, and epidemiology of malaria by Afridi, M. K. *Proc. 5th Intern. Congr. Trop. Med. Malaria* (Istanbul, Turkey, August–September, 1953) (In press)
56. Huff, C. G., Marchbank, D. F., Saroff, A. H., Scrimshaw, P. W., and Shiroishi, T., *J. Natl. Malaria Soc.*, **9,** 307 (1950)
57. Inoki, S., *Med. J. Osaka Univ.*, **2,** 45 (1951)
58. Jaswant Singh, Ray, A. P., and Nair, C. P., *Nature*, **172,** 122 (1953)
59. Jeffrey, G. M., Wolcott, G. B., Young, M. D., and Williams, D., Jr., *Am. J. Trop. Med. Hyg.*, **1,** 917 (1952)
60. Laird, M., *J. Parasitol.*, **37,** 183 (1951)
61. Laird, M., *J. Parasitol.*, **39,** 357 (1953)
62. Levaditi, C., and Schoen, R., *Compt. Rend. Soc. biol.*, **109,** 343 (1931)
63. Lewert, R. M., *Science*, **107,** 250 (1948)
64. Lewert, R. M., *J. Infectious Diseases*, **91,** 125 (1950)
65. Manresa, M., Jr., *J. Parasitol.* **39,** 452 (1953)
66. Manwell, R. D., *Am. J. Hyg.*, **43,** 1 (1946)
67. Manwell, R. D., *J. Parasitol.* **37,** 319 (1951)
68. Marchiafava, E., and Celli, A., *Fortschr. Med.*, **3,** 787 (1885)
69. McGhee, R. B., *J. Parasitol.*, **35,** 545 (1949)
70. McGhee, R. B., *J. Infectious Diseases*, **88,** 86 (1951)
71. Mer, G. G., and Goldblum, N., *Nature*, **159,** 444 (1947)
72. Miller, W. W., *U. S. Public Health Service, Hyg. Lab. Bull., No. 46*, 5 (1908)
73. Mudrow, L., *Z. Tropenmed. Parasitol.*, **1,** 113 (1949)
74. Mudrow, L., and Reichenow, E., *Zool. Jahrbücher* (*Anatomie*), **70,** 129 (1949)
75. Mulligan, H. W., and Sommerville, T., *Indian J. Malariol.*, **1,** 329 (1947)
76. Muniz, J. (To be published)
77. Muniz, J., Soares, R., and Batista, S., *Rev. brasil. malariol.*, **3,** 345 (1951)
78. Nicolajev, B. P., *Med. Parasitol. Parasitic Diseases* (*U.S.S.R.*), **8,** 191 (1939)
79. Oberlé, G., *Bull. soc. pathol. exotique*, **38,** 27 (1945)
80. Paraense, W. L., *Mem. inst. Oswaldo Cruz*, **45,** 813 (1947)
81. Perez-Reyes, R., *J. Parasitol.*, **39,** 603 (1953)
82. Raffaele, G., *Riv. malariol.*, **16,** 413 (1937)
83. Raffaele, G., *Riv. malariol.*, **31,** 1 (1952)
84. Ray, H. N., *Proc. Natl. Inst. Sci. India*, **15,** 241 (1949)
85. Rodhain, J., *Bull. soc. Pathol. exotique*, **19,** 828 (1926)
86. Rodhain, J., *Acta Biol. Belg.*, **1,** 118 (1941)

87. Rodhain, J., *Ann. Soc. Biol. Med. trop.*, **28**, 39 (1948)
88. Rodhain, J., *Bull. Acad. Roy. Med. Belgique*, **14**, 17 (1949)
89. Rodhain, J., *Ann. soc. belge méd. trop.*, **32**, 275 (1952)
90. Rodhain, J., *Ann. soc. belge méd. trop.*, **33**, 285 (1953)
91. Russell, P. F., *Malaria: basic principles briefly stated*, Basil Blackwell & Mott, Ltd., Oxford, England, 210 pp., 1952
92. Schingarew, A., *Arch. sci. biol., St. Petersburg*, **12**, 181 (1907)
93. Schmidt, L. H., *Proc. 5th Intern. Congr. Trop. Med. Malaria* (In press)
94. Schwetz, J., *Arch. inst. Pasteur Algérie*, **8**, 289 (1930)
95. Schwetz, J., *Zentr. Bakteriol. Parasitenk., Abt. I*, **130**, 111 (1933)
96. Sergent, Edmond, *Arch. inst. Pasteur Algérie*, **27**, 211 (1949)
97. Shortt, H. E., and Bray, R. S., *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, 2 (1952)
98. Shortt, H. E., Bray, R. S., and Cooper, W., *Trans. Roy. Soc. Trop. Med. Hyg.* (In press)
99. Shortt, H. E., and Cooper, W., *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, 427 (1948)
100. Shortt, H. E., Fairley, N. H., Covell, G., Shute, P. G., and Garnham, P. C. C., *Brit. Med. J.*, **II**, 1006 (1949)
101. Shortt, H. E., and Garnham, P. C. C., *Nature*, **161**, 126 (1948)
102. Shortt, H. E., and Garnham, P. C. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, 285 (1948)
103. Shortt, H. E., and Garnham, P. C. C., *Brit. Med. J.*, **I**, 1225 (1948)
104. Shortt, H. E., Garnham, P. C. C., Covell, G., and Shute, P. G., *Brit. Med. J.*, **I**, 547 (1948)
105. Shortt, H. E., Garnham, P. C. C., and Malamos, B., *Brit. Med. J.*, **I**, 192 (1948)
106. Taliaferro, W. H., and Taliaferro, L. G., *J. Infectious Diseases*, **80**, 78 (1947)
107. Thompson, P. E., and Huff, C. G., *J. Infectious Diseases*, **74**, 48 (1944)
108. Tonkin, I. M., and Hawking, F., *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, 407 (1947)
109. Van den Berghe, L., Vincke, I. H., and Chardome, M., *Ann. soc. belge méd. trop.*, **30**, 79 (1950)
110. Van Riel, J., Hiernaux-L'Hoest, D., and Hiernaux-L'Hoest, J., *Parasitology*, **41**, 270 (1951)
111. Vincke, I. H., and Lips, M., *Ann. soc. belge med. trop.*, **28**, 1, 97 (1948)
112. Vincke, I. H., Parent, M., Berteaux, M., and de Meulemaere, J., *Bull. assoc. Congolaise anciens étud. univ. Liege*, **5**, 30 (1945)
113. Vincke, I. H., and Peeters, E., *Ann. soc. belge méd. trop.*, **33**, 87 (1953)
114. Weathersby, A. B., *J. Infectious Diseases*, **91**, 198 (1952)
115. Wilcox, A., Tomlinson, T. H., Jr., and Ballard, M. B., *J. Natl. Malaria Soc.*, **9**, 249 (1950)
116. Wolfson, F., and Winter, M. W., *Am. J. Hyg.*, **44**, 273 (1946)
117. Yoeli, M., and Wall, W. J., *Nature*, **168**, 1078 (1951)
118. Zuckermann, A., *J. Infectious Diseases*, **79**, 1 (1946)

MECHANISMS OF ANTIBACTERIAL ACTION[1]

By W. W. Umbreit

Merck Institute for Therapeutic Research, Rahway, N. J.

The availability of the review of Heilman (1) covering the clinical, therapeutic, and nutritional literature through 1952, and the review of Work (2) on the biochemistry of the antibiotics through 1951, somewhat simplifies the present review and permits a partial return to the subject of mode of action of the antibiotics, last reviewed in this series by Brownlee in 1951 (3). In addition, there are several available reviews devoted specifically to considerations of the mode of action of antibiotics (4 through 10). At the present juncture, therefore, the reviewer feels that it is sensible to consider what advances have been made recently in understanding of mode of action of antibiotics, to record those changes which have occurred in our knowledge of the chemical nature of antibiotic substances and to list, as briefly as possible, the newer types of antibiotics being reported. These will be considered, first, under the names of the better known antibiotics; the less well-known will be separated on the basis of antibacterial, antifungal, or antiviral activity and further divided on the basis of knowledge of their chemical character. While there is, therefore, a great deal in this review which is not specifically directed toward the mode of action of antibiotics, the present requirements of research workers in this field are better met even if merely by reference to associated work. Comment by the reviewer will be restricted to studies on the mode of action of antibiotics. Clinical studies have been excluded.

Penicillin

The conclusion that penicillin acts by inhibiting an early stage of ribosenucleic acid synthesis (8) has not been essentially altered, and during the year there have been no further reports on the mode of action. Studies on biosynthesis of penicillin (11 to 14) show its origin from formate, acetate, less from lactate, none from CO_2. Its production under practical conditions (15), in synthetic media (16 to 18), and with pigment-free strains (19) have been described. Mutation toward better yield by the use of uranium nitrate (20) and a new type of penicillin called cephalosporin N (21, 22), which appears to be an α-amino adipyl derivative of the essential core of the penicillin molecule (23), have been reported. Sodium benzoyl penicillin stimulates seed germination at 5 units per ml. or less (24) and, of all species, the guinea pig appears to be especially sensitive to penicillin (25, 26). Penicillin, together with some 30 other antibiotics, has been tested for effectiveness against *Clostridium botulinum* (27) with a view toward food preservation. The en-

[1] The survey of the literature pertaining to this review was concluded in December, 1953.

hancement of penicillin action by cobalt, sometimes reported, may be related to the inhibition of respiration in *Salmonella* sp. by cobalt (28). Penicillinase is strikingly protected against heat inactivation by gelatin or the constituents of complex media (29).

A great deal of study (see 30) has been devoted to combinations of antibiotics with penicillin. The following are recent references (31 to 37), since it is clearly impossible to summarize the results in a short space. The amount of penicillin required to inhibit growth has been reported to be increased 4 to 8 fold by the addition to the medium of complex natural materials ("baseamine") (38) and similar results were obtained with streptomycin, the tetracyclenes, chloramphenicol, and bacillin.

Streptomycin

The properties of this drug have been specifically reviewed in detail (39, 40) and the relation between chemical structure and mode of action discussed (41). The locus of action of streptomycin, which previously could only be specified as an unknown reaction between oxalacetate and pyruvate (3, 7, 9, 10) is gradually being clarified. Its action on pyruvate metabolism was again shown indirectly in a different type of bacterial system (42, 43) but the need of a more direct approach has apparently been met by the isolation of a new metabolic intermediate (2-phospho-4-hydroxy-4-carboxyadipic acid) and the demonstration that not only is it an active metabolic intermediate, but also that it is formed in the bacteria only when pyruvate plus a four-carbon dicarboxy acid are present and that its formation is completely prevented by streptomycin (44). This now permits more direct study of the sensitive reaction. Because previous methods of measurement were dependent upon indirect indications of the reaction, such as extent and rate of respiration, the relationship of the new intermediate to the rate of respiration was sometimes not obvious, and particularly the killing effect was not always related to the rate or amount of oxygen uptake (45). But such studies (45) led to an observation of considerable interest in that the rate of killing in resting cell suspensions was greater when they were metabolizing exogenous substrate. This is about all the information that has any special bearing upon the mode of action of the drug reported during 1953. Nevertheless, a great many other studies on streptomycin were made which bear mentioning. The locus of action in preventing the growth of clostridia from spores is on the vegetative cell, not on the spore (46). The beneficial effect of streptomycin in radiation sickness has been explored (47 to 50).

A large proportion of the studies on streptomycin are devoted to a study of resistance (and dependence) which, by now, has been definitely established as a genetic change in the resistant or dependent mutant. In fact, streptomycin resistance may be induced in sensitive *Hemophilus influenzae* by extracts of the resistant strain (51). The increased rate of mutation to resistance by exposure to ultraviolet light has been further studied (52, 53) and a factor inhibiting the resistant strain liberated by autolysis from bac-

teria killed by streptomycin has been reported (54). Dependence upon streptomycin has always been most puzzling, particularly since no one has yet been able to demonstrate the utilization of streptomycin during growth. A streptomycin-dependent mutant of *Bacillus cereus* apparently requires the antibiotic in the synthesis of cytochrome (55). A heat stable factor (presumably not streptomycin) which will replace the streptomycin requirement of the dependent strain has been found in the dialyzed fraction of bovine blood serum (56). Strains resistant to streptomycin accumulate more ribonucleic acid than sensitive strains (57) and possess enhanced ability to synthesize amino acids in the presence of streptomycin (58). Papers on cross resistance (59 to 61) and on the mechanism of resistance as shown towards structural metabolites (62 to 64) cannot be discussed here and it is doubtful whether these tell one much about resistance to antibiotics, except to emphasize the complexity of the information.

Relation to Tuberculosis

The mycobacteria require special mention, but the present report is but a minor selection from the wealth of material available. Antitubercular activity is present in spermine (NH_2-$(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$) if a tissue substance is present. This substance is a contaminant of the albumin added in most modern media and possesses enzymatic activity, bringing about the rapid oxidative deamination of spermine or spermidine, but differs from the usual amine oxidase (65 to 67). Both isonicotinic hydrazide (INH) and 1-isonicotinyl-2-isopropyl hydrazide inhibit diamine oxidase and guanidine deaminase in the bacteria (68). The latter has a marked effect in inhibiting mammalian monamine oxidase (69). When fed, these drugs reduce the activity of tyramine oxidase (70). While INH is inactivated in Dubos medium when alkaline (71), and by ketones (72) which has been proposed as a mode of action (73), the most reasonable mode of action of INH, that of antagonizing vitamin B_6 (74, 75), cannot apparently be applied to the tubercle organism in which such a relationship does not hold (76). The INH analogue of diphosphopyridine nucleotide (DPN) can be prepared (77) but its significance in the action of INH as an antitubercular agent has yet to be assessed. INH shows activity in animal leprosy (78, 79). INH-D-glucuronolactone is considered perhaps better than INH (80) but it is evident that, since INH-resistant tuberculosis of mice responds to streptomycin and thiosemicarbazones, the mechanism of action of the three drugs is different (81) in spite of the possession of certain common structural features. They have also been studied in the electron microscope (82). The relation of structure to antitubercular activity has been discussed (83) and the design of laboratories for the safe handling of the experimental infection has received attention (84).

Chloramphenicol

The mode of action of this substance is unknown and various observations on its action are not yet sufficiently well oriented to permit any conclusions

(8, 10). It is further claimed that a site of action is interference with the synthesis of indole from anthranilic acid (85). When fed to rats, it decreases liver xanthine oxidase, but is without effect on intestinal xanthine oxidase (86). Various decomposition products stimulate the growth of *E. coli* and those convertible into *p*-nitro-benzaldehyde are effective antagonists of the antibiotic (87, 88). Chloramphenicol does not interfere with the action of penicillin (89) in practice. Analogues have been prepared and studied, particularly as possible antivirals (90 to 97), without striking success. The dynamics of synthesis during fermentation has been explored (98).

The Tetracyclene Group (Aureomycin, Terramycin, Tetracyclene)

The mode of action of this group is not known and observations on phosphorylation uncoupling (8), are not adequate to explain its action.

Reasons for nomenclature changes in this group are described (99) with the present names as: tetracyclene, chlortetracyclene (aureomycin), oxytetracyclene (terramycin). As a group, the drugs combine with ions (100), and Mn^{++} will reverse aureomycin inhibition of the bacterial cell-free nitroreductase (101). At relatively high doses (125 μg./ml.), oxytetracyclene was not toxic in tissue culture (102), but 5 μg./ml. was bacteriostatic to *Actinomyces* (103). Curiously, the serum level of chlortetracyclene or oxytetracyclene could be increased as much as tenfold by the simultaneous administration of citrate, malic acid, tartaric acid, malonic acid, etc. (104). Oxytetracylene (and streptomycin, penicillin, or neomycin) were anticoagulants only when used in concentrations much higher than can be achieved *in vivo* (105). Comparison of the bacterial spectrum of the tetracyclene group with other antibiotics, including erythromycin, has been reported (106). As somewhat of a side issue but of interest, the feeding of sulfaguanidine, oxytetracyclene, and penicillin to mice completely abolished all urease activity (107), whose origin, therefore, must be from gastrointestinal bacteria rather than the tissue of the mammal. Tetracyclene may be prepared by chemical treatment of chlortetracyclene (108) or oxytetracyclene (109). Further chemical studies on these substances have been reported (110, 111) but it appears that the molecule as a whole, rather than the presence or orientation of individual groups, determines the potency of these drugs (112).

Erythromycin, Ilotycin

The mode of action is not known. This is a "broad spectrum" antibiotic (essentially inactive against many gram-negative bacteria (113 to 115) but has some effect against the larger viruses (113) and apparently acts on susceptible bacteria only during multiplication (116). On acid degradation the antibiotic yields dimethylamine and 3-dimethylamino-4-desoxy-5-methylaldopentose (117). Details of assay, absorption, etc., will be found in the following references (118 to 122). Resistance develops with fair rapidity (115, 123) and there is a cross resistance to carbomycin (magnamycin) (115, 124). Combination with other drugs has been studied (125).

Carbomycin, Magnamycin

The mode of action is not known but is obviously related to erythromycin. The substance is primarily effective against gram positive organisms (126 to 130) and some viruses; it is of low toxicity (131) and contains an unusual branched chain dexoxy sugar, called mycarose (132).

Actithiazic Acid

This substance [4-thiazolidone-2-caproic acid (133, 134)], produced by *Streptomyces* sp. and primarily active against mycobacteria, appears to interfere with biotin synthesis in these organisms (135). This seems to be the same substance produced by *Streptomyces cinnamonensis* (136, 137) although this species is claimed to produce a polypeptide antibiotic termed cinnamycin (138). Of further interest is the synthesis of the actithiazic acid (134, 139, 140) and its relatives, some of which proved more active than the original antibiotic.

The Toxic Peptides

In this series of substances, there are two whose mode of action is known. Antimycin A acts as a pseudoirreversible inhibitor of succinoxidase demonstrable *in vivo* as well as *in vitro* (141, 142). Chemical studies show that antimycic acid, a degradation product of antimycin A is N-(3-aminosalicyloyl)-L-threonine (143). The Polymyxin peptides appear to cause a release of soluble cell constituents into the medium (144). The substances are not toxic to tissue cultures (145), are adsorbed more readily by sensitive than resistant cells (146), adsorption is inhibited by Mg^{++} ion (147), by anionic detergents, etc., (148). Physical (149) and pharmacological (150) properties have been recorded. The development of resistance to polymyxin B is dependent upon the amino acid content of the growth medium (151). The mode of action of the remainder of the peptides is not known. Neomycin has been the subject of a book (152) and subsequent studies report on characteristics (153), assay (154, 155), production (156), and factors influencing *in vitro* activity (157). Bacitracin is resolvable into as many as 10 components (158, 159), the molecular weight of the principle component being 1460 to 1470 (160, 161), and apparently contains 2 carboxyls, one α-amino group, one ornithine residue, one histidine glyoxaline, and the Σ-amino group of lysine is not free (160, 162). The peptide contains one each of D-phenylalanine, D-glutamic acid, D-ornithine, L-leucine, L-cysteine, L-lysine with two each of aspartic acid and L-isoleucine (163). The antibacterial action is increased by cationic detergents (164). Improved assay methods have been described (165).

Actinomycins have been studied with respect to chemical separation (166 to 168) and composition (169). Several of the actinomycins contain L-threonine, sarcosine, L-proline, D-valine, and L-N-methylvaline in varying proportions. Two types also contain D-*allo*-isoleucine. Spores of *Clostridium botulinum* are reported to be appreciably less resistant to heat when sus-

pended in food containing small amounts of subtilin (170), normally considered a surface active agent (171). The Nisin family of peptides contain lanthionine and an isomer of cystathionine (172, 173).

Structures of Miscellaneous Antibiotics

In this entire group little is known of the mode of action except what can be surmised from the chemical structure. Mycomycin (174) is related to allene and is (—)3,5,7,8-*n*-tri-decatetraene-10,12-diyonic acid (175 to 177). Drosophilin C is also a polyacetylene compound (178) as is the antibiotic from *Clitocybe diatreta* now identified as suberamic acid ene-diyne (179). A structure for puromycin (achromycin) has been proposed (180) and one of its degradation products, 3-amino-D-ribose has been synthesized (181). This structure is related to that of nubularine which is 9-D-ribosylpurine (182).

In addition to methyl gallate, ethyl gallate has appeared among the antibiotics (183, 184). The *Gliocladium* sp. antibiotics have been identified. Aurantiogliocladin is 2,3-dimethoxy-5,6-dimethyl-*p*-benzoguinone (185) and has been synthesized (186), while rubogliocladin is the corresponding quinhydrone and gliorosein is the dihydro derivative of aurantiogliocladin. Related, but more complicated structures occur in nidulin and ustin which contain chlorine (187). Among the lichen antibiotics (reviewed 188), vulpic acid, pinastric acid, and calycin and usnic acid have been identified (189) and the structure of usnic acid confirmed (190).

Antibiotics studied during 1953 whose structures are less well-known were: Rhodomycin (191), chartreusin (192), viridication (193), a basic crystalline antitubercular (194), and a material from seaweed extracts thought to be a brominated phenol (195).

Chemical structures for the following antibiotics reported on in 1953 are not known, nor is the mode of action indicated in any case: thioaurin (196), flaveolin (197), sarcidin (198), collinomycin (199), amicetin (200), rubromycin (201), rhodocidin (202), actiline or soframycin (203), synnematin (204), fluvomycin (205), and an unnamed, toxic antibiotic (206).

The following are primarily antifungal agents whose importance in therapy is probably on the increase. Their mode of action is not known. Gladiolic acid is 4-methyoxy-5-methyl-*o*-phthalaldehyde-3-carboxylic acid (207) and is related to mycophenolic acid (208) and the esters of *p*-hydroxybenzoic acid (209). The following antifungal agents are not characterized chemically: candicidin (210, 211), trichomycin (212), moldin and phaeofacin (213), chromin (214), rotaventin (215), phalamycin (216), and helixin (217). Aureomycin but not terramycin stimulates growth of *Candida albicans* (218) and protection is afforded mice by fungicidin (219).

Fumagillin acts upon the Nosema disease of bees by arresting or destroying the infection during its intracellular phase (220).

Antiviral Agents

The following materials are primarily antiviral agents. Their mode of action is not known. At this point, it is perhaps pertinent to emphasize that the nature of virus disease differs so markedly from bacterial infections that perhaps all they have in common is their transmissibility. It is, therefore, by no means certain that an effective antibiotic or chemotherapeutic agent will be found for them. There are now, however, more encouraging signs that chemotherapy may eventually be possible. At the outset, there seem to be three general possibilities of action against the viruses: first, interference with adsorption on or penetration into susceptible cells; second, interference with the development of the virus within the cell; third, interference with the release of formed virus from the cell.

In addition to the effects of the tetracyclenes, chloramphenicol, and magnamycin in certain virus diseases, agents have been reported which markedly affect the course of viral infections in the infected animal without possessing *in vitro* antiviral activity or any discernible antibacterial activity. A substance, termed helenine, active in this manner against neurotropic viruses (221, 222) has been purified (223) and an apparently similar material (224, 225) has been reported, all from *Penicillium* cultures. Penicillin itself has an *in vitro* antiviral action against the virus of Newcastle disease (226) and Netropsin is active against vaccinial infections by the W R strain (227). An antiviral microbial product has also been reported (228). *In vitro* inactivation of vaccinia in cell culture has been related to glucuronic acid (229). Some progress has been made in synthetic antivirals, especially among derivatives of benzimidazole (230, 231, 232).

New techniques should be mentioned. Application of electrophoresis on paper to crude antibiotic preparations permits the determination of whether they are acidic, basic, or amphoteric and their approximate isoelectric point as a guide to subsequent purification (233). A replicate plate method of screening antibiotic producing organisms permits rapid recognition of the antibiotic producers and a partial antimicrobial spectrum of a culture even before the culture is actually isolated (234). The plaque method for determining virus particles, used extensively in the study of bacteriophage, may now be applied more generally to viruses infecting animal tissue (235).

LITERATURE CITED

1. Heilman, F. R., *Ann. Rev. Microbiol.*, **7**, 219–44 (1953)
2. Work, T. S., *Ann. Rev. Biochem.*, **21**, 431–58 (1952)
3. Brownlee, G., *Ann. Rev. Microbiol.*, **5**, 197–208 (1951)
4. Gale, E. F., *Proc. Intern. Congr. Biochem., 2nd Congr.*, 5–20, (Paris, France, 1952)
5. Gros, F., and Macheboeuf, M., *Proc. Int. Congr. Biochem., 2nd Congr.*, 101–23, (Paris, France, 1952)
6. Hotchkiss, R. D., *Ann. Rev. Microbiol.*, **II**, 183–214 (1948)

7. Umbreit, W. W., *Proc. Intern. Congr. Biochem.*, **2nd. Congr.** 63–77, (Paris, France, 1952)
8. Umbreit, W. W., *Pharmacol. Revs.*, **5,** 275–84 (1953)
9. Umbriet, W. W., and Oginsky, E. L., *J. Mt. Sinai, Hosp., N. Y.*, **19,** 175–84 (1951)
10. Wyss, O., Smith, G. N., Hobby, G. L., Oginsky, E. L., and Pratt, R., *Bact. Revs.*, **17,** 17–94 (1953)
11. Perret, C. J., *J. Gen. Microbiol.*, **8,** 195–97 (1953)
12. Martin, E., Berky, J., Godzesky, C., Miller, P., Tome, J., and Stone, R. W., *J. Biol. Chem.*, **203,** 239–50 (1953)
13. Tome, J., Zook, H. D., Wagner, R. B., Stone, R. W., *J. Biol. Chem.*, **203,** 251–55 (1953)
14. Gordon, M., Pan. S. C., Virgona, A., and Numerof, P., *Science*, **118,** 43 (1953)
15. Davey, V. F., and Johnson, M. J., *Appl. Microbiol.*, **1,** 208–11 (1953)
16. Mortimer, D. C., and Johnson, M. J., *J. Am. Chem. Soc.*, **74,** 4098–4102 (1952)
17. Hosler, P., and Johnson, M. J., *Ind. Eng. Chem.*, **45,** 871–74 (1953)
18. Soltero, F. V., and Johnson, M. J., *Appl. Microbiol.*, **1,** 52–57 (1953)
19. Anderson, R. F., Whitmore, L. M., Brown, W. E., Peterson, W. H., Churchill, B. W., Roegner, F. R., Campbell, T. H., Backus, M. P., and Stauffer, J. F., *Ind. Eng. Chem.*, **45,** 768–73 (1953)
20. Gattani, M. L., *Science*, **116,** 596–97 (1952)
21. Abraham, E. P., Newton, G. G. F., Crawford, K., and Burton, H. S., *Nature*, **171,** 343 (1953)
22. Heatley, N. G., and Florey, H. W., *Brit. J. Pharmacol.*, **8,** 252–58 (1953)
23. Newton, G. G. F., and Abraham, E. P., *Nature*, **172,** 395 (1953)
24. von Weihe, K., *Chem. Eng.*, **60,** 203–4 (1953)
25. Ambrus, C. M., Sideri, C. N., Johnson, G. C., and Harrisson, J. W. E., *Antibiotics & Chemotherapy*, **2,** 521–27 (1952)
26. Stevens, K. M., Gray, I., *Antibiotics & Chemotherapy*, **3,** 731–40 (1953)
27. Andersen, A. A., Michener, H. D., and Olcott, H. S., *Antibiotics & Chemotherapy*, **3,** 521–26 (1953)
28. Pital, A., Stafseth, H. J., and Lucas, E. H., *Science*, **117,** 459–60 (1953)
29. Manson, E. E. D., and Pollock, M. R., *J. Gen. Microbiol.*, **8,** 163–67 (1953)
30. Jawetz, E., and Gunnison, J. B., *Pharmacol. Revs.*, **5,** 175–92 (1953)
31. Purcell, E. M., Wright, S. S., and Finland, M., *Proc. Soc. Exptl. Biol. Med.*, **82,** 124–31 (1953)
32. Finland, M., and Wilcox, C., *Proc. Soc. Exptl. Biol. Med.*, **83,** 605–8 (1953)
33. Jawetz, E., and Gunnison, J. B., *Antibiotics & Chemotherapy*, **2,** 243–48 (1952)
34. Miyahara, B. T., Cariker, K., and Clapper, W. E., *J. Lab. Clin. Med.*, **51,** 550–58 (1953)
35. Klein, M., and Schorr, S. E., *J. Bact.*, **65,** 454–65 (1953)
36. Chabbert, Y., *Ann. inst. Pasteur*, **84,** 545–61 (1953)
37. Henneberg, G., *Arzneimittel-Forsch.*, **3,** 25–26 (1953)
38. Foster, J. W., and Pittillo, R. F., *J. Bact.*, **65,** 361–68 (1953)
39. Waksman, S. A., *Science*, **118,** 259–66 (1953)
40. Waksman, S. A., *Antibiotics & Chemotherapy*, **3,** 333–46 (1953)
41. Umbreit, W. W., *Trans. N. Y. Acad. Sci.*, **15,** 8–11 (1952)
42. Barkulis, I. L., *J. Bact.*, **65,** 337–43 (1953)
43. Zebovitz, E., and Moulder, J. W., *J. Bact.*, **65,** 344–47 (1953)

44. Umbreit, W. W., *J. Bact.*, **66**, 74–81 (1953)
45. Paine, R. F., and Clark, L. S., *Science*, **118**, 73–74 (1953)
46. Wynne, E. S., Collier, R. E., and Mehl, D. A., *J. Bact.*, **64**, 883–86 (1952)
47. Marston, R. Q., Ruth, H. J., and Smith, W. W., *Proc. Soc. Exptl. Biol. Med.*, **83**, 289–91 (1953)
48. Smith, W. W., Smith, F., Ruth, J., Canter, H. Y., and Grenan, M. M., *Am. J. Physiol.*, **172**, 351–58 (1953)
49. Gonshery, L., Marston, R. Q., and Smith, W. W., *Am. J. Physiol.*, **172**, 359–64 (1953)
50. Marston, R. Q., Gonshery, L., Alderman, I. M., and Smith, W. W., *Am. J. Physiol.*, **172**, 365–71 (1953)
51. Alexander, H. E., and Leidy, G., *J. Exptl. Med.*, **97**, 17–31 (1953)
52. Mefferd, R. B., *Proc. Soc. Exptl. Biol. Med.*, **81**, 123–24 (1952)
53. Haas, F. L., Wyss, O., Berger, H., and Stone, W. S., *J. Bact.*, **65**, 354 (1953)
54. Olitzki, A. L., *Nature*, **171**, 132–33 (1953)
55. Schaeffer, P., *Biochim. et Biophys. Acta*, **9**, 563–71 (1952)
56. Sevag, M. G., and Rosanoff, E. I., *Arch. Biochem. and Biophys.*, **45**, 633–34 (1953)
57. Beljanski, M., *Compt. rend.*, **236**, 1102–4 (1953)
58. Sevag, M. G., and Rosanoff, E. I., *J. Bact.*, **63**, 243–51 (1952)
59. Szybalski, W., *Antibiotics & Chemotherapy*, **3**, 1095–1103 (1953)
60. Pagano, J. F., Weinstein, M. J., and Donovick, R., *Proc. Soc. Exptl. Biol. Med.*, **79**, 359–63 (1952)
61. Fusillo, M. H., Romansky, M. J., and Kuhns, D. M., *Antibiotics & Chemotherapy* **3**, 35–40 (1953)
62. Davis, B. D., and Maas, W. K., *Proc. Natl. Acad. Sci.*, **38**, 775–85 (1952)
63. Yaniv, H., and Davis, B. D., *J. Bact.*, **66**, 238 (1953)
64. Davis, B. D., *Proc. Natl. Acad. Sci.*, **39**, 363–66 (1953)
65. Hirsch, J. G., *J. Exptl. Med.*, **97**, 323–25 (1953)
66. Hirsch, J. G., *J. Exptl. Med.*, **97**, 327–43 (1953)
67. Hirsch, J. G., *J. Exptl. Med.*, **97**, 345–55 (1953)
68. Zeller, E. A., Barsky, J., Fouts, J. R., Kirchheimer, W. F., and Van Orden, L. S. *Experientia*, **8**, 349–50 (1952)
69. Zeller, E. A., and Barsky, J., *Proc. Soc. Exptl. Biol. Med.*, **81**, 459–61 (1952)
70. Viollier, G., Quiring, E., and Staub, H., *Helv. Chim. Acta*, **36**, 724–30 (1953)
71. Pansy, F. E., Koerber, W. L., Stander, H., and Donovick, R., *Am. Rev. Tuberc.*, **68**, 284–285 (1953)
72. Schaefer, W. B., *Am. Rev. Tuberc.*, **68**, 273–76 (1953)
73. Neuberg, C., and Forrest, I. S., *Arch. Biochem. and Biophys.*, **45**, 237–38 (1953)
74. Yoneda, M., Kato, N., and Okajima, M., *Nature*, **170**, 803 (1952)
75. Yoneda, M., and Asano, N., *Science*, **117**, 277–79 (1953)
76. Boone, I. U., and Woodward, K. T., *Proc. Soc. Exptl. Biol. Med.*, **84**, 292–96 (1953)
77. Zatman, L. J., Kaplan, N. O., Colowick, S. P., and Ciotti, M. M., *J. Am. Chem. Soc.*, **75**, 3293–94 (1953)
78. Cruickshank, J. C., *Lancet*, **II**, 624–25 (1953)
79. Levaditi, C., Vaisman, A., and Chaigneau-Erhard, H., *Compt. rend.*, **236**, 549–51 (1953)
80. Sah, P. P. T., *J. Am. Chem. Soc.*, **75**, 2512–13 (1953)

81. Hirsch, J., *Naturwissenschaften*, **39**, 525–26 (1952)
82. Brieger, E. M., Cosslett, V. E., and Glauert, A. M., *Nature*, **171**, 211–12 (1953)
83. Di Marco, A., *Antibiotics & Chemotherapy*, **3**, 807–8 (1953)
84. Solotorovsky, M., Robinson, H. J., and Kniazuk, M., *Am. Rev. Tuberc.*, **68**, 212–19 (1953)
85. Bergmann, E. D., and Sicher, S., *Nature*, **170**, 931–32 (1952)
86. Swendseid, M. E., Swanson, A. L., and Bethell, F. H., *J. Biol. Chem.*, **201**, 811–15 (1953)
87. Smith, G. N., *Arch. Biochem. and Biophys.*, **40**, 314–22 (1952)
88. Smith, G. N., and Worrel, C. S., *J. Bact.*, **65**, 313–17 (1953)
89. Ahern, J. J., Burnell, J. M., and Kirby, W. M. M., *Proc. Soc. Exptl. Biol. Med.*, **79**, 568–71 (1952)
90. Phillips, A. P., *J. Am. Chem. Soc.*, **74**, 6125–27 (1952)
91. Phillips, A. P., *J. Am. Chem. Soc.*, **75**, 3621–22 (1953)
92. Collins, R. J., Ellis, B., Hansen, S. B., Mackenzie, H. S., Moualim, R. J., Petrow, V., Stephenson, O., and Sturgeon, B., *J. Pharm. and Pharmacol.*, **4**, 693–710 (1952)
93. Rebstock, M. C., and Moore, A. C., *J. Am. Chem. Soc.*, **75**, 1685–88 (1953)
94. Taylor, E. P., *J. Pharm. and Pharmacol.*, **5**, 254–56 (1953)
95. Van Der Meer, S., Kofman, H., and Veldstra, H., *Rec. trav. chim.*, **72**, 236–46 (1953)
96. Sensi, P., and Fagioli, O., *Gazz. chim. ital.*, **83**, 73–77 (1953)
97. Billet, D., and Marnay, C., *Compt. rend.*, **235**, 706–8 (1952)
98. Legator, M., and Gottlieb, D., *Antibiotics & Chemotherapy*, **3**, 809–17 (1953)
99. Editorial, *Antibiotics & Chemotherapy*, **3**, 659–62 (1953)
100. Albert, A., *Nature*, **172**, 201 (1953)
101. Saz, A. K., and Slie, R. B., *J. Am. Chem. Soc.*, **75**, 4626–27 (1953)
102. Schrek, R., *Antibiotics & Chemotherapy*, **3**, 783–87 (1953)
103. Howell, A., *Antibiotics & Chemotherapy*, **3**, 378–81 (1953)
104. Eisner, H. J., Stirn, F. E., Dornbush, A. C., and Oleson, J. J., *J. Pharmacol. Exptl. Therap.*, **108**, 442–49 (1953)
105. Braude, A. I., Davey, P., and Siemienski, J., *Proc. Soc. Exptl. Biol. Med.*, **83**, 160 (1953)
106. Welch, H., Randall, W. A., Reedy, R. J., and Kramer, J., *Antibiotics & Chemotherapy*, **2**, 693–96 (1952)
107. Dintzis, R. Z., and Hastings, A. B., *Proc. Natl. Acad. Sci. U. S.*, **39**, 571–78 (1953)
108. Boothe, J. H., Morton, J., Petisi, J. P., Wilkinson, R. G., and Williams, J. H., *J. Am. Chem. Soc.*, **75**, 4621 (1953)
109. Conover, L. H., Moreland, W. T., English, A. R., Stephens, C. R., and Pilgrim, F. J., *J. Am. Chem. Soc.*, **75**, 4622–23 (1953)
110. Boothe, J. H., Kushner, S., Petisi, J., and Williams, J. H., *J. Am. Chem. Soc.*, **75**, 3261–63 (1953)
111. Boothe, J. H., Kushner, S., and Williams, J. H., *J. Am. Chem. Soc.*, **75**, 3263–64 (1953)
112. Regna, P. P., *Trans. N. Y. Acad. Sci.*, **15**, 12–17 (1952)
113. Powell, H. M., Boniece, W. S., Pittenger, R. C., Stone, R. L., and Culbertson, C. G., *Antibiotics & Chemotherapy*, **3**, 165–82 (1953)
114. Haight, T. H., and Finland, M., *Proc. Soc. Exptl. Biol. Med.*, **81**, 175–83 (1952)

115. Finland, M., Wilcox, C., Wright, S. S., and Purcell, E. M., *Proc. Soc. Exptl. Biol. Med.*, **81**, 725–29 (1952)
116. Haight, T. H., and Finland, M., *Proc. Soc. Exptl. Biol. Med.*, **81**, 188–93 (1952)
117. Clark, R. K., Jr., *Antibiotics & Chemotherapy*, 663–71 (1953)
118. Higgens, C. E., Pittenger, R. C., and McGuire, J. M., *Antibiotics & Chemotherapy*, **3**, 50–54 (1953)
119. Josselyn, L. E., and Sylvester, J. C., *Antibiotics & Chemotherapy*, **3**, 63–66 (1953)
120. Ziegler, D. W., and McGuire, J. M., *Antibiotics & Chemotherapy*, **3**, 67–70 (1953)
121. Kirby, W. M. M., Maple, F. M., and O'Leary, B., *Antibiotics & Chemotherapy*, **3**, 473–77 (1953)
122. Ford, J. H., Prescott, G. E., Hinman, J. W., and Caron, E. L., *Anal. Chem.*, **25**, 1195 (1953)
123. Haight, T. H., and Finland, M., *Proc. Soc. Exptl. Biol. Med.*, **81**, 183–88 (1952)
124. Fusillo, M. H., Noyes, H. E., Pulaski, E. J., and Tom, J. Y. S., *Antibiotics & Chemotherapy*, **3**, 581–86 (1953)
125. Powell, H. M., Culbertson, C. G., Boniece, W. S., Streightoff, F., Baker, L. A., Metcalf, H. B., *Antibiotics & Chemotherapy*, **3**, 701–8 (1953)
126. English, A. R., Field, M. F., Szendy, S. R., Tagliani, N. J., and Fitts, R. A., *Antibiotics & Chemotherapy*, **2**, 678–88 (1952)
127. Tanner, F. W., English, A. R., Lees, T. M., and Routien, J. B., *Antibiotics & Chemotherapy*, **2**, 441–43 (1952)
128. English, A. R., Mullady, H. E., and Fitts, R. A., *Antibiotics & Chemotherapy*, **3**, 94–98 (1953)
129. Seneca, H., and Ides, E., *Antibiotics & Chemotherapy*, **3**, 117–21 (1953)
130. Wong, S. C., James, C. G., Finlay, A., *Antibiotics & Chemotherapy*, **3**, 741–49 (1953)
131. Gardocki, J. F., P'an, S. Y., Rapuzzi, A. L., Fanelli, G. M., and Timmens, E. K., *Antibiotics & Chemotherapy*, **3**, 55–62 (1953)
132. Regna, P. P., Hochstein, F. A., and Wagner, R. L., Jr., *J. Am. Chem. Soc.*, **75**, 4625–26 (1953)
133. Schenck, J. R., and De Rose, A. F., *Arch. Biochem. and Biophys.*, **40**, 263–69 (1952)
134. Pennington, F. C., Celmer, W. D., McLamore, W. M., Bogert, V. V., and Solomons, I. A., *J. Am. Chem. Soc.*, **75**, 109–14 (1953)
135. Grundy, W. E., Whitman, A. L., Rdzok, E. G., Rdzok, E. J., Hanes, M. E., and Sylvester, J. C., *Antibiotics & Chemotherapy*, **2**, 399–408 (1952)
136. Maeda, K., Okami, Y., Kosaka, H., Taya, O., and Umezawa, H., *J. Antibiotics (Japan)*. **5**, 572–73 (1952)
137. Umezawa, H., Oikawa, K., Okami, Y., and Maeda, K., *J. Bact.*, **66**, 118–19 (1953)
138. Benedict, R. G., Dvonch, W., Shotwell, O. L., Pridham, T. G., and Lindenfelser, L. A., *Antibiotics & Chemotherapy*, **2**, 591–94 (1952)
139. Clark, R. L., and Schenck, J. R., *Arch. Biochem. and Biophys.*, **40**, 270–76 (1952)
140. McLamore, W. M., Celmer, W. D., Bogert, V. V., Pennington, F. C., Sobin, B. A., and Solomons, I. A., *J. Am. Chem. Soc.*, **75**, 105–8 (1953)
141. Potter, V. R., and Reif, A. E., *J. Biol. Chem.*, **194**, 287–97 (1952)
142. Reif, A. E., and Potter, V. R., *Cancer Research*, **13**, 49–57 (1953)
143. Tener, G. M., van Tamelen, E. E., and Strong, F. M., *J. Am. Chem. Soc.*, **75**, 3623–25 (1953)

144. Newton, B. A., *J. Gen. Microbiol.*, **9**, 54–64 (1953)
145. Metzger, J. F., Fusillo, M. H., and Kuhns, D. M., *Antibiotics & Chemotherapy*, **2**, 227–28 (1952)
146. Few, A. V., and Schulman, J. H., *Nature*, **171**, 644–45 (1953)
147. Newton, B. A., *Nature*, **172**, 160–61 (1953)
148. Rhodes, R. E., Vila, O. A., and Ferlauto, R. J., *Antibiotics & Chemotherapy*, **3**, 509–12 (1953)
149. Few, A. V., and Schulman, J. H., *Biochem. J.* (*London*) **54**, 171–76 (1953)
150. Brownlee, G., Bushby, S. R. M., and Short, E. I., *Brit. J. Pharmacol.*, **7**, 170–88 (1952)
151. Haas, G. J., and Sevag, M. G., *Arch. Biochem. and Biophys.*, **43**, 11–24 (1953)
152. Waksman, S. A., *Neomycin; Nature, Formation, Isolation, and Practical Application*, (Rutgers University Press, New Brunswick, New Jersey, 233 pp., 1953)
153. Hamre, D. M., Pansy, F. E., Lapedes, D. N., Perlman, D., Bayan, A. P., and Donovick, R., *Antibiotics & Chemotherapy*, **2**, 135–41 (1952)
154. Abbey, A., *Antibiotics & Chemotherapy*, **2**, 528–31 (1952)
155. Dutcher, J. D., Hosansky, N., and Sherman, J. H., *Antibiotics & Chemotherapy*, **3**, 534–36 (1953)
156. Dulmage, H. T., *Appl. Microbiol.*, **1**, 103–6 (1953)
157. Schoenhard, D. E., and Stafseth, H. J., *Antibiotics & Chemotherapy*, **3**, 41–49 (1953)
158. Newton, G. G. F., and Abraham, E. P., *Biochem. J.* (*London*) **53**, 597–603 (1953)
159. Craig, L. C., Weisiger, J. R., Hausmann, W., and Harfenist, E. J., *J. Biol. Chem.*, **199**, 259–66 (1952)
160. Craig, L. C., Hausmann, W., and Weisiger, J. R., *J. Biol. Chem.* **200**, 765–73 (1953)
161. Porath, J., *Acta Chem. Scand.*, **6**, 1237–48 (1952)
162. Newton, G. G. F., and Abraham, E. P., *Biochem. J.* (*London*) **53**, 604–12 (1953)
163. Craig, L. C., Hausmann, W., and Weisiger, J. R., *J. Biol. Chem.*, **199**, 865–71 (1952)
164. Bruce, C. B., and Mitchell, L., *J. Am. Pharm. Assoc., Sci. Ed.*, **41**, 654–56 (1952)
165. Hadfield, A. S., *Antibiotics & Chemotherapy*, **2**, 590 (1952)
166. Brockmann, H., and Pfennig, N., *Hoppe-Seyler's Z. physiol. Chem.*, **292**, 77–88 (1953)
167. Brockmann, H., and Gröne, H., *Naturwissenschaften*, **40**, 222–23 (1953)
168. Brockmann, H., Linge, H., and Gröne, H., *Naturwissenschaften*, **40**, 224 (1953)
169. Brockmann, H., Bohnsack, G., and Gröne, H., *Naturwissenschaften*, **40**, 223–24 (1953)
170. LeBlanc, F. R., Devlin, K. A., and Stumbo, C. R., *Chem. Eng. Progr.*, **49**, 181–84 (1953)
171. Sacks, L. E., *Antibiotics & Chemotherapy*, **2**, 79–85 (1952)
172. Berridge, N. J., Newton, G. G. F., and Abraham, E. P., *Biochem. J.* (*London*) **52**, 529–35 (1952)
173. Newton, G. G. F., and Abraham, E. P., *Nature*, **171**, 606 (1953)
174. Celmer, W. D., and Solomons, I. A., *J. Am. Chem. Soc.*, **74**, 2245–48 (1952)
175. Celmer, W. D., and Solomons, I. A., *J. Am. Chem. Soc.*, **74**, 1870–71 (1952)
176. Celmer, W. D., and Solomons, I. A., *J. Am. Chem. Soc.*, **75**, 1372–76 (1953)
177. Celmer, W. D., Solomons, I. A., *J. Am. Chem. Soc.*, **75**, 3430–35 (1953)

178. Anchel, M., *Arch. Biochem. and Biophys.*, **43**, 127–35 (1935)
179. Anchel, M., *J. Am. Chem. Soc.*, **75**, 4621–22 (1953)
180. Waller, C. W., Fryth, P. W., Hutchings, B. L., and Williams, J. W., *J. Am Chem. Soc.*, **75**, 2025 (1953)
181. Baker, B. R., and Schaub, R. E., *J. Am. Chem. Soc.*, **75**, 3864–65 (1953)
182. Lofgren, N., and Luning, B., *Acta Chem. Scand.*, **7**, 225 (1953)
183. Little, J. E., Foote, M. W., Rogers, W. I., and Johnstone, D. B., *Antibiotics & Chemotherapy*, **3**, 183–91 (1953)
184. Johnstone, D. B., Foote, M. W., Rogers, W. I., and Little, J. E., *Antibiotics & Chemotherapy*, **3**, 203–7 (1953)
185. Vischer, E. B., *J. Chem. Soc.* (*London*) 815–20 (1953)
186. Baker, W., McOmie, J. F. W., and Miles, D., *J. Chem. Soc.* (*London*) 820–22 (1953)
187. Dean, F. M., Robertson, A., Roberts, J. C., and Raper, K. B., *Nature*, **172**, 344 (1953)
188. Klosa, J., *Die Pharmazie*, **5**, 435–42 (1953)
189. Klosa, J., *Pharmazie*, **7**, 687–88 (1952)
190. Barton, D. H. R., and Brunn, T., *J. Chem. Soc.* (*London*) 603–9 (1953)
191. Brockmann, H., and Borchers, I., *Chem. Ber.* **86**, 261–69 (1953)
192. Leach, B. E., Calhoun, K. M., Johnson, L. E., Teeters, C. M., and Jackson, W. G., *J. Am. Chem. Soc.*, **75**, 4011–12 (1953)
193. Cunningham, K. G., and Freeman, G. G., *Biochem. J.* (*London*) **53**, 328–32 (1953)
194. McCormick, M. H., and Hoehn, M. M., *Antibiotics & Chemotherapy*, **3**, 718–20 (1953)
195. Mautner, H. G., Gardner, G. M., and Pratt, R., *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 294–96 (1953)
196. Bolhofer, W. A., Machlowitz, R. A., and Charney, J., *Antibiotics & Chemotherapy*, **3**, 382–84 (1953)
197. Takahashi, B., *J. Antibiotics* (*Japan*) [A] **6**, 11–20 (1953)
198. Takeuchi, T., Nitta, K., and Umezawa, H., *J. Antibiotics* (*Japan*) [A] **6**, 31–32 (1953)
199. Brockmann, H., and Renneberg, K., *Naturwissenschaften*, **40**, 166–67 (1953)
200. DeBoer, C., Caron, E. L., and Hinman, J. W., *J. Am. Chem. Soc.*, **75**, 499–500 (1953)
201. Brockmann, H., and Renneberg, K., *Naturwissenschaften*, **40**, 59–60 (1953)
202. Charney, J., Machlowitz, R. A., Roberts, W. S., and Fisher, W. P., *Antibiotics & Chemotherapy*, **3**, 788–92 (1953)
203. Decaris, M. L.-J., *Ann. pharm. franç.*, **11**, 44–46 (1953)
204. Olson, B. H., Jennings, J. C., and Frank, A. J., *Science*, **117**, 76–78 (1953)
205. Carvajal, F., *Antibiotics & Chemotherapy*, **3**, 765–72 (1953)
206. Eisenman, E., Minieri, P. P., Abbey, A., Charlebois, J., Moncrieff-Yeates, M., and Rigler, N. E., *Antibiotics & Chemotherapy*, **3**, 385–92 (1953)
207. Grove, J. F., *Biochem. J.* (*London*) **54**, 664–73 (1953)
208. Delmotte, P., and Delmotte-Plaquee, J., *Nature*, **171**, 344 (1953)
209. Siegel, M., *Antibiotics & Chemotherapy*, **3**, 478–80 (1953)
210. Lechevalier, H., Acker, R. F., Corke, C. T., Haenseler, C. M., and Waksman, S. A., *Mycologia*, **45**, 155–71 (1953)

211. Kligman, A. M., and Lewis, F. S., *Proc. Soc. Exptl. Biol. Med.*, **82,** 399–404 (1953)
212. Hosoya, S., Komatsu, N., Soeda, M., Yuwaguchi, T., and Sonoda, Y., *J. Antibiotics (Japan)*, **5,** 564–66 (1952)
213. Maeda, K., Okami, Y., Taya, O., and Umezawa, H., *J. Antibiotics (Japan)*, **5,** 465 (1952)
214. Wakaki, S., Akanabe, S., Hamada, K., and Asahina, T., *J. Antibiotics (Japan)*, **5,** 680–81 (1952)
215. Hosoya, S., Komatsu, N., and Soeda, M., *J. Antibiotics (Japan)*, **5,** 453–58 (1952)
216. Brown, R., and Hazen, E. L., *Antibiotics & Chemotherapy*, **3,** 818–21 (1953)
217. Leben, C., Stessel, G. J., and Keitt, G. W., *Mycologia*, **44,** 159–69 (1952)
218. Huppert, M., MacPherson, D. A., and Cazin, J., *J. Bact.*, **65,** 171–76 (1953)
219. Brown, R., Hazen, E. L., and Mason, A., *Science*, **117,** 609–10 (1935)
220. Bailey, L., *Nature*, **171,** 212–13 (1953)
221. Shope, R. E., *J. Exptl. Med.*, **97,** 601–25 (1953)
222. Shope, R. E., *J. Exptl. Med.*, **97,** 627–38 (1953)
223. Shope, R. E., *J. Exptl. Med.*, **97,** 639–50 (1953)
224. Powell, H. M., Culbertson, C. G., McGuire, J. M., Hoehn, M. M., and Baker, L. A., *Antibiotics & Chemotherapy*, **2,** 432–34 (1952)
225. Powell, H. M., and Culbertson, C. G., *Proc. Soc. Exptl. Biol. Med.*, **83,** 161–63 (1953)
226. Kohn, A., *Proc. Soc. Exptl. Biol. Med.*, **82,** 612–16 (1953)
227. Schabel, F. M., Jr., Laster, W. R., Jr., Brockman, R. W., and Skipper, H. E., *Proc. Soc. Exptl. Biol. Med.*, **83,** 1–3 (1953)
228. Groupe, V., Pugh, L. H., and Levine, A. S., *Proc. Soc. Exptl. Biol. Med.*, **80,** 710–14 (1952)
229. McCrea, J. F., and Duran-Reynals, F., *Science*, **118,** 93–95 (1953)
230. Tamm, I., Folkers, K., and Horsfall, F. L., Jr., *J. Exptl. Med.*, **98,** 219–27 (1953)
231. Tamm, I., Folkers, K., and Horsfall, F. L., Jr., *J. Exptl. Med.*, **98,** 229–43 (1953)
232. Tamm, I., Folkers, K., Shunk, C. H., Heyl, D., and Horsfall, F. L., *J. Exptl. Med.*, **98,** 245–59 (1953)
233. King, N. K., and Doery, H. M., *Nature*, **171,** 878–79 (1953)
234. Lechevalier, H. A., and Corke, C. T., *Appl. Microbiol.*, **1,** 110–12 (1953)
235. Dulbecco, R., *Proc. Natl. Acad. Sci. (U. S.)*, **38,** 747–52 (1952)

GENETICS OF MICROORGANISMS[1,2]

S. SPIEGELMAN AND O. E. LANDMAN
Department of Bacteriology, University of Illinois, Urbana, Illinois

INTRODUCTION

The primary purpose of a review in the present series is perhaps best served by a complete and well-organized presentation of the relevant literature. To add on to this task an attempt at a critical integrative analysis of the available information would require an amount of space which might well exceed that assigned to the entire present volume.

With reference to the criterion of complete coverage the authors are aware of certain omissions, some deliberate, others unavoidable. Thus, the cognate and somewhat frenetic field of bacterial cytology has been omitted (with some relief) since it is discussed elsewhere in the present volume. Further, we have not quoted any information contained in the *Microbial Genetics Bulletin* in deference to the stated policies of this journal which derives much of its usefulness from its informality. Finally, we have encountered our share of journals missing, at the bindery, or unavailable for other reasons.

Three books have appeared in the last year containing extensive discussions of various aspects of microbial genetics. Braun (71) gives a very readable account of bacterial genetics in a style which should be accessible to the uninitiated. Ephrussi's book (136) summarizes his own investigations on cytoplasmic inheritance in yeast, Sonneborn's on protozoa, and the studies on the ascomycete *Podospora anserina*. Luria (275) presents a lucid and pedagogically useful account of viral genetics in his text, which is devoted to a unified survey of virology. In addition to these, mention may be made of four symposium volumes (106, 140a, 278a, 441a), all international in scope, which are devoted either entirely, or in large part, to problems of microbial and viral inheritance.

The almost explosive rapidity with which microbial genetics is developing has resulted in a prolixity of terms and consequent complications not easily resolved. The difficulty generated is well-exemplified by a recent paper in which four luminaries of the field have focused the bright white light of their combined talents on the terminological complexities of microbial genetics (137). The result is remarkable for its brevity.

Our survey of the literature of microbial genetics has left us with a strong impression of the tremendous vitality and creativeness in this field. It seems

[1] The survey of literature pertaining to this review was completed in December, 1953.

[2] The following abbreviations have been used in this chapter: DNA (desoxyribonucleic acid); PAB or PABA (*p*-aminobenzoic acid); RNA (ribonucleic acid); POB (*p*-hydroxybenzoic acid).

likely that microbial genetics will continue for some time to be a fruitful source of information pertinent to problems of fundamental biological interest.

PHENOGENETICS

Genetic Analysis of Intermediate Metabolism

Reviews.—Various aspects of the field of genetic analysis of intermediate metabolism have been reviewed by Adelberg (3), Davis (109), and Mitchell (294). In the following paragraphs, contributions are classed according to the areas of metabolism to which they are relevant.

Amino acids.—Ames, Mitchell & Mitchell (7) have isolated and identified three accumulation products from histidineless mutants of *Neurospora* and have provided good evidence that their phosphorylated counterparts are intermediates in the biosynthesis of histidine from sugar. A pathway of ornithine synthesis characteristic of *Escherichia coli* has been elucidated by Vogel (436) who studied a mutant blocked between α-N-acetylornithine and ornithine. Two successive intermediates were isolated which fit into a consistent biosynthetic scheme.

Davis and his co-workers (109, 110, 111) have continued their elucidation of aromatic biosynthesis in *E. coli*. They succeeded in isolating and characterizing a sequence of four intermediates: 5-dehydroquinic acid, 5-deydroshikimic acid, shikimic acid, and phosphoshikimic acid. Evidence for the direct conversion of anthranilic acid to hydroxyanthranilic acid in *Aspergillus* is reported (338).

Common intermediates in the synthesis of valine and pantoic acid have been uncovered by Maas & Vogel (282). Ketovaline is the branching point of the biosynthetic pathways. The deficiency of an isoleucineless mutant of *E. coli* has been identified as being attributable to lack of transaminase B, an enzyme involved in the formation of isoleucine, valine, leucine, norleucine, norvaline, and glutamic acid (3a, 370). The mutant does contain transaminase A which catalyzes transaminations between glutamic acid, phenylalanine, tyrosine, tryptophan, and aspartic acid (370). A lysineless mutant of *E. coli* has been shown to be lacking an enzyme which decarboxylates diaminopimelic acid (118). The pathway of lysine biosynthesis in *Aspergillus* appears to differ from both the *Neurospora* and the *E. coli* pathways (338).

Evidence for four genetic classes of methionine mutants in yeast has been obtained (335). The ability of methionine auxotrophs to utilize adenine as well as such compounds as thiomethyl adenosine (383) adds confirmatory evidence to the recently uncovered relationships between adenine and methionine metabolism. Evidence for a pathway leading from aspartic acid to homoserine to threonine has been obtained with mutants of *E. coli* by Hirsch & Cohen (201, 201a). Umbarger (426) has also studied aspects of this pathway and comes to the conclusion that the pathway of threonine proposed on the basis of earlier work (426a) must be abandoned. No alternative biosynthetic scheme consistent with all the data could be postulated.

Complex interactions in methionine, adenine, threonine, thiazole, and serine biosynthesis have also been uncovered in *Neurospora* (126). Amino acid inhibitions in 100 isolates of *E. coli* have been studied (368). Inhibition by valine in strain K-12 can be reversed by a single gene mutation (284). Similarly, arginine inhibition of a lysine mutant and lysine inhibition of an arginine mutant in *Neurospora* are eliminated by mutation (395). In a mutant of *E. coli* a multiple amino acid requirement on glucose medium disappears on acetate; glucose inhibits acetate oxidation (425). In a leucineless mutant of *E. coli*, the growth inhibition observed in the presence of isoleucine and valine may be reversed by leucine dipeptides (200).

Wild strains of *Erwinia aroideae* produce potato rot. Amino acid requiring mutants of this organism may lose their virulence, however, depending upon the availability of free amino acids in the potato (157). In large scale experiments involving 67 auxotrophs of *Salmonella typhimurium*, Gowen *et al.* (164a) reached the conclusion that there was no consistent correlation between auxotrophy or specific metabolite requirements on the one hand and virulence to mice to immunizing power on the other.

Davis (108) and Katagiri & Sato (228) have described a paradoxical situation in *E. coli*, in which a phenylalanine requiring mutant accumulates phenylalanine in the medium. Investigation revealed that the primary accumulation product was a nonutilizable substance which was converted in acid medium to phenylpyruvic acid and eventually to phenylalanine. Phenylpyruvic acid has growth factor activity, and thus an autocatalytic growth phenomenon is observed.

Purine and pyrimidine metabolism.—Purine (73) and pyrimidine (314) mutants of *Aerobacter aerogenes* have been isolated, and their growth factor requirements and growth characteristics have been determined.

Vitamin, hormone, and porphyrin biosynthesis.—Partridge & Giles (328) have examined 15 inositol auxotrophs of *Neurospora* for their biochemical characteristics. Of the 15 strains, 11 are known to be allelic, and, except for a temperature mutant, all behave identically. Granick, Bogorad and coworkers (67, 68, 167) have continued their brilliant elucidation of chlorophyll biosynthesis in *Chlorella*. Hematoporphyrin-9 and monovinyl-monohydroxyethyl-porphyrin-9 have been identified as precursors of protoporphyrin-9. A mutant of *Chlamydomonas* which grows normally in the dark but requires acetate in the light (34) has been described. A mutant of *Micrococcus aureus* has been obtained which requires hemin for aerobic growth. Anaerobically-grown cells lack catalase and cytochrome-*a* and -*b* activity, but the protein moieties of these enzymes are probably present. Evidence for an independent synthesis of the prosthetic groups of catalase and cytochrome is given (220, 221, 222). The primary lesion of a mendelian character for cytochrome deficiency in yeast (355) was shown to be a slow synthesis of glycine which results in a depressed biosynthesis of the prosthetic groups of catalase and cytochrome (471). Mendelian characters controlling cytochrome are also described for *Neurospora* (295).

Birch, Donovan & Moewus (65) have delineated the steps leading to isorhamnitin, a mating hormone of *Chlamydomonas eugametos*. Eight classes of genetically female mutants unable to copulate with males were obtained. With their aid two pathways leading to isorhamnitin, one involving phenylalanine and tyrosine and the other *meso*-inositol were implicated. The two pathways appear to join at an unknown compound related to quercetin. Intermediate isolation and syntrophic experiments provided data consistent with the biosynthetic scheme proposed.

Pigment biosynthesis.—A start has been made in analyzing the biosynthetic pathways of some pigments related to penicillin (16, 17).

Inorganic metabolism.—Seven mutants concerned with nitrogen metabolism were obtained in *Neurospora*. In some of these strains enzymatic deficiencies were found for glutamic dehydrogenase and nitrate reductase, one forming an enzyme inhibitor (385). Mutants of *Azotobacter* blocked between N_2 and NH_2 were obtained by the use of the penicillin method (168). Reduction of selenite to selenium in a mutant of *Neurospora* has been shown to be linked to methionine metabolism (473).

Fatty acid synthesis.—Two steps in the biosynthesis of unsaturated fatty acids in *Neurospora* have been elucidated by the use of biochemical mutants. As a result, a synthetic scheme separating unsaturated from saturated fatty acid synthesis has been proposed (254).

Oxidative metabolism.—A deficiency in α-ketoglutarate oxidase in the phycomycete *Blastocladiella emersonii* has been found to be associated with an inability to form resistant sporangia (85).

Raut (355) isolated a genic mutant which resembles the cytoplasmic "petites" in being a respiratory deficient. In crosses to either wild type or "petites" a 2:2 ratio of mutant to wild phenotype is obtained. An analogous case of a genic mutant which phenotypically mimics a cytoplasmic variant ("poky") has been found by Mitchell and his collaborators in *Neurospora* (295).

Much of the work on respiratory mutants in yeast (199, 287, 350, 388, 389) and *Neurospora* (185, 424) deals with cytoplasmic rather than genic variants and will be detailed in the section on cytoplasmic factors.

Generalized effects.—Three auxotrophs of *Neurospora* grown on limiting quantities of their required metabolites were found to mimic the enzymatic changes found in zinc deficiency. A fourth mutant showed the reverse reaction (313).

Morphological and Resistance Mutants

Morphological mutations.—A peculiar morphology in a *Neurospora* mutant has been shown to be due to inability to retain water (242). Light depresses tyrosinase activity and melanogenesis in a black mutant of *Neurospora* (379).

Sussman and his collaborators (415, 417) found that mutants of the slime mold *Dictyostelium discoideum* were unable to carry out the morphogenetic

cycle by themselves but were morphogenetically competent in mixtures. It seems likely that continued work along the lines opened up by these investigators not only will make a genetic investigation of this group of organisms possible but will also provide data highly pertinent to fundamental aspects of morphogenesis.

Mutation to auxotrophy in *E. coli* is sometimes correlated with the production of mucoid morphology. The composition of the polysaccharide of various such strains was examined and found to be the same (64). Mutants of tobacco mosaic virus can be differentiated by their physical and immunological properties and by the disease syndromes they cause (384).

Mutation to bacteriophage resistance.—The consequences of bacterial mutation to phage resistance have been elucidated in several studies. In streptococci, a higher amino acid requirement is associated with phage resistance (327). Jesaitis & Goebel (163, 223, 224) have isolated and purified a receptor for coli phages T3, 4 and 7 from wild strains. This substance is not present in strains resistant to these three phages. Analogous results are reported by Beumer (62). A case of complete bacterial cross resistance against a certain phage and a colicine has been reported by Fredericq (150). The possibility of charting the bacterial surface by the use of specific receptors is indicated by results obtained with mutants resistant to phages and bacteriocins [colicin-like substances] (215).

A special class of heritable changes is associated with the lysogenization of sensitive bacteria. Thus, the lysogenic state confers on the lysogenic bacterium immunity to further infection with the carried phage. Further, it may entail change in colony (212) or cell (179) morphology. Finally, dramatic changes in virulence have been linked to the presence of prophage (36, 172, 173, 174, 197, 198).

Mutants resistant to physical conditions.—Mutations from the mesophilic state to the thermophilic state are either very rare or nonexistent (96). Mutants resistant to γ-radiation have been obtained (155).

Drug Resistance

Genetic control of drug resistance.—Cavalli-Sforza & Lederberg (91) have made an excellent critical survey of the genetics of resistance to bacterial inhibitors of various kinds. The same general area has been reviewed from somewhat different points of view by Dean & Hinshelwood (112) and Abraham (1a). Yudkin (472) has proposed a theory of drug resistance in bacteria which seeks to compromise between genetic and nongenetic mechanisms. Unfortunately, several features make it difficult to accept the Yudkin model. In the first place, no distinction is drawn between genotype and phenotype. Secondly, one must postulate an inordinate number of genes controlling resistance to implement his mechanism.

Compelling support for a classical genetic interpretation of drug resistance phenomena derives from work of Cavalli & Maccacaro (90). These authors examined progeny from crosses between strains of *E. coli* with vary-

ing degrees of resistance to chloromycetin. The results clearly indicate the involvement of a polygenic system. On crossing highly resistant mutants to sensitives, recombinants showing all degrees of resistance between those of the parents were obtained. Polygenes determining resistance were linked to ordinary chromosome markers. Finally, recombination between independent resistant mutants gave rise to organisms more resistant than either parent strain.

Experiments employing infective transfer of hereditary material also provide supporting evidence for the genetic nature of resistance. Thus Alexander & Leidy (5) have induced resistance to streptomycin in sensitive strains of *Hemophilus influenzae* by exposure to DNA[2] obtained from resistant strains. Similar results have been obtained in transduction experiments with *Salmonella* spp. as summarized by Zinder (480). One step mutations leading to the resistance to isoniazid (421) and a folic acid antagonist (142) have been demonstrated. *Pasteurella pestis* was found to mutate to streptomycin resistance at a rate of 5×10^{-11} (158).

In his studies on the origin of streptomycin resistance Mitchison (296, 297) noted that independent isolates of resistant organisms could be differentiated into four physiological types. Since members of a particular resistant clone were always of the same physiological type, the differences between types could be plausibly explained only if one assumed their origin to be mutational. An attempt to establish that the loss of resistance to chloramphenicol is due to selection of sensitive mutants is made inconclusive by the methods employed (190). Mutation to resistance to a variety of inhibitory agents and conditions is reportedly correlated with mutation to a lactose-positive condition (83).

On the basis of the independence of mutational events the genetic view of the origin of drug resistance predicts that the simultaneous use of a pair of drugs should be more effective in preventing the development of resistant strains than the separate or successive use of the same two drugs. This genetic prediction is verified in certain instances, provided both drugs are used at a high level (234, 255, 420). Drug antagonism may be observed when one or both drugs are used at a bacteriostatic level. Bacteriostasis slows down bactericidal action and therefore increases the chance for mutation (234, 420). Not all cases of drug interactions appear to be interpretable in these terms (174a, 175).

Biochemical mechanisms of drug resistance.—There are in principle a variety of ways by means of which a cell can overcome the effects of an inhibitory agent. Amongst these we may cite the following: (*a*) the development of an enzyme system which destroys the agent; (*b*) the development of an alternative pathway not susceptible to the agent; (*c*) modification of the relevant enzyme so that it no longer has affinity for the agent; (*d*) a shift in metabolic equilibria which tends to offset the effects of the agent; (*e*) the establishment of permeability barriers.

The finding of rare mutants which produce penicillinase (418) and others

which produce chloramphenicol reductase (292) confirms the existence of mechanism (*a*). The existence of mechanism (*b*) has been postulated to account for a very complex series of observations involving streptomycin resistance (365). Davis (107) has studied an interesting case in which the inhibiting agent, being a normal metabolite, is endogenously produced, while the metabolite which reverses the inhibiting affect is exogenous. The recovery of mutants resistant to the endogenous inhibitor made it possible to rule out mechanism (*e*) or variations of mechanism (*d*) leading to an increased synthesis of the reversing metabolite. Davis suggests that the evidence available is most easily explained in terms of mechanism (*c*). The same mechanism was used to explain the behavior of sulfonamide-resistant strains which exhibited an increased requirement for PABA.[3] Recently, however, Yaniv & Davis (469) found that increased PABA requirement is not an obligatory consequence of mutation to sulfonamide resistance. Mechanism (*d*) can account for the resistance of a strain of *Streptococcus fecalis* to amethopterin (4-amino-N^{10}-methylpteroyl glutamic acid), a citrovorum factor antagonist. The mutant organism produces 100 times as much citrovorum factor as does its parental strain (74). An analogous situation was uncovered in the case of a streptomycin-resistant mutant which exhibited increased activity of the pantothenate synthesizing enzyme which is reported to be inhibited by streptomycin (466).

Eagle (131) provides evidence that mechanism *e* may function in natural resistance of bacteria to penicillin. Using S^{35} labelled penicillin this author shows that highly sensitive organisms concentrate penicillin more efficiently than resistant strains. However, resistant mutants retain the penicillin accumulating power of their sensitive parent strains. Sussman & Bradley (416) have succeeded in eliminating mechanisms *a* and *e* as explanations for the resistance of mutant yeast strains to arsenate and azide.

An interesting case, probably involving mechanisms *b* or *d*, is analyzed in the study of a mutant of *Lactobacillus casei* resistant to 2,6-diaminopurine (135b). The mutant uses 2,6-diamino-purine-riboside much better than its parent strain, while the latter uses adenine more efficiently. A mutant resistant to 6-mercaptopurine (135, 135a) also revealed a constellation of altered metabolic properties.

The remarkable flexibility of the mutational potentiality of microorganisms is exhibited in their ability to become dependent upon inhibitory agents. In the past year, two cases of mutation to penicillin dependence (33, 34, 418) have been reported. Bryson *et al.* (77a) have isolated isoniazid dependent strains of *Mycobacterium ranae*. Mutant strains of *Micrococcus pyogenes* show growth stimulation in the presence of penicillin and chloromycetin (419). A better understanding of streptomycin dependence may ultimately emerge from the finding that an unidentified substance in bacterial extracts replaces the streptomycin necessary for the growth of streptomycin-dependent strains of *E. coli* (382).

Phenotypic effects associated with drug resistance.—It is not surprising that

mutation to drug resistance should in some instances be accompanied by changes in other biological properties of the organisms. Various instances of such associated changes have been reported. Among the most interesting from the practical point of view is the manifestation of resistance to other antibiotics in organisms selected for resistance to any one (141, 286, 351, 419). The most frequent constellation of cross-resistance is that which is directed against the bacterial polypeptide antibiotics (419).

Observations have been reported which indicate that the acquisition of resistance to streptomycin is accompanied by an increase in the RNA^2 content of the cells (46, 47, 48, 392). Associated changes involving virulence (467) and penicillinase production (35) have been reported in mutants selected for resistance to streptomycin. Employment of the proper medium permits the distinction in terms of colony color of streptomycin-resistant cells on the one hand from sensitive and dependent cells on the other. This provides a visual method for the detection of back mutants (290).

Cytoplasmic Inheritance and Phenotypic Variation

The papers to be discussed document the existence of an almost continuous spectrum of autonomy, extending from cases in which there is virtually complete self-determination of cytoplasmic elements to others where cytoplasmic autonomy is highly evenescent or nonexistent. In between there are many cases which were aptly described by Lederberg in a recent review (248a) as "straddling the persistence of genetic variation and the transience of physiological response."

Cytoplasmic factors.—Further studies on the removal of plastids from strains of *Euglena* have revealed that aureomycin treatment can give rise to colorless clones. These clones are not always stable, however, and several have been observed to revert. A yellow cell type has been recovered from cultues kept at 36°C. which gives rise to mixed clones composed of colorless, green, and yellow cells (362). Dusi (130) reports that light protects against the mutagenic effects of streptomycin and that the presence of the drug prevents cells bleached by growth in the dark from making pigment when placed in the light.

The phenomenon of mate-killing in interspecific crosses between Variety 4 and Variety 8 animals of *Paramecium aurelia* was discovered and investigated intensively by Siegel (383a) and Levine (257, 257a, 257b). From the results of amicronucleate×micronucleate crosses between varieties, Levine (257a) reached the conclusion that, formally, mate-killing can be attributed to incompatibility of the nucleus of each variety with the cytoplasm of the other. In some instances, an amicronucleate Variety 8 animal fertilized with a Variety 4 nucleus survived and was transformed into a Variety 4 animal.

Mate-killing by Variety 8 is caused by the presence of a cytoplasmic particle similar to kappa which is called mu (257b, 383a). Mating between different stocks of *Paramecium* containing mu revealed a "peck order" of killing potency. Killing was in part a function of mu concentration. While the

maintenance of mu, like that of kappa, is gene-dependent, the specific dominance relationships between mu-carrying stocks are probably not gene controlled. The effects of mu and kappa are independently demonstrable (257b).

The mate-killing phenomenon was also investigated at the enzymological level, but no metabolic differences between the varieties were found which could account for the killing (257).

Preer and his colleagues (342, 343, 344) have made a detailed study of the correlation between cytoplasmic particles and the killer phenotype. They recognized the existence of three kinds of cytoplasmic entities: the presence of kappa leads to the production and liberation of paramecin; cells containing mu will kill sensitives only if they mate; pi particles are morphologically like kappa or mu but they produce no identifiable phenotypic effect. In animals possessing the usual killer phenotype a cytological distinction was possible amongst the kappa particles. Certain of them contained a refractile region, and are therefore referred to as "brights." Centrifugation experiments established a correlation between the killer-property and the "brights."

A striking and simple relationship between gene dosage and the cytoplasmic factor kappa has been discovered by Chao (92). In a given mating type, the homozygote KK contains twice as many kappa particles as the heterozygote Kk. Organisms of mating type 7, however, contain twice as many kappa particles as the animals of corresponding genotype of mating type 8. These relationships hold despite the fact that the absolute number of particles varies rather drastically with the conjugation state of the organisms.

Knowledge of the effect of gene dosage on the number of kappa particles provided the opportunity to examine the extent of the multiplicative autonomy of kappa. By following the kappa count in cells of genetic constitution kk after autogamy in a heterozygote, Chao (92) was able to demonstrate that kappa loss was very gradual. His data show that kappa is able to increase between 2^{10}- and 2^{11}-fold in the absence of the dominant K. He further demonstrated a delay of about eight generations before the kappa particles rose from the level characteristic of the heteozygotes to that which obtains in the homozygote KK.

The inactivation of kappa by x-rays has been found to be accelerated by oxygen (160). A survey of a large number of compounds uncovered one purine (2,6-diamino-purine) and two antibiotics [chlortetracycline (aureomycin) and oxytetracycline (terramycin)] which were effective in "curing" paramecia of kappa (409). In connection with Chao's (92) observations, it is of interest to note that in the presence of adenine, which antagonizes 2,6-diamino-purine, much higher yields of kappa are obtained. Increases up to 425 per cent were observed.

A system having many parallels to the "petite" case in yeast, the maternal mutant "poky," was discovered in *Neurospora* by Mitchell and his co-workers. Further work (185, 424) on this strain reveals that the level of

cytochrome-*c* in the "poky" strain is above normal, whereas its content of cytochrome oxidase and succinic oxidase is suppressed. These differences are most marked in early growth. An interesting feature of the "poky" character is the presence of a hitherto unknown enzyme which destroys cytochrome-*c*. The respiration of "poky" seems to be mediated via a flavin enzyme which is insensitive to cyanide. Striking changes in the cytochrome pigments of another "poky-like" cytoplasmic variant and two gene mutants analogous to "poky" and of combinations of the nuclear and cytoplasmic mutants are described (295).

The ability of chelating agents to release mitochondria-like bodies from yeast has been noted (291). Ultraviolet radiation is reported to disrupt mitochondria in yeast (377). Bodies claimed to be mitochondria on the basis of reactions to the Nadi reagent, tetrazolium, and Janus Green B, have been counted in living and dividing yeast cells (305). The range of the number of particles per cell is from 1 to 18, with a median near 6. Another investigation (42) of the number of Nadi-reacting bodies in yeast yielded an average value of 13.2 particles per cell. The author states that the distribution is not Poissonian and finds a correlation between the cell size and the number of these particles. The same author (43) shows that if the reaction is carried out with living, anaerobically grown organisms, only 2 to 5 per cent of the cells give a positive test. In aerobically grown yeast, however, 40 per cent of the cells react positively. In both cases either all of the particles of a cell stain, or none do. It is of the greatest interest to establish with certainty the number and distribution during division of such elements, in view of the relevance of this information to an understanding of the spontaneous and induced production of petite mutants.

Ephrussi (136) and his collaborators continue to make fundamental contributions to our understanding of the cytoplasmic factor which they discovered in yeast, the loss of which gives rise to a respiratory deficient strain called "petite." Slonimski (388) summarizes in detail the results of his extensive researches into the biochemistry of respiratory enzyme formation and the properties of "petites." Slonimski & Hirsch (199, 390) examined the enzymatic content of "petites." The pattern obtained was compared with that observed in anaerobically grown wild type. The cytoplasmic variants showed complete deficiency in enzymes known to be located on particles. The modification in content of the nonparticulate enzymes (malic, alcohol and isocitric dehydrogenase; aconitase and fumarase) was roughly similar to the change observed in anaerobically grown wild type.

Ogur, Lindegren & Lindegren have reported data which imply that a cytoplasmic "mutation" in yeast may assume a dominant form (322). Similar observations were discussed by Ephrussi at the Bellagio Genetics Congress (personal communication). In both cases a "petite" was isolated which on crossing with wild type yielded four "petite" segregants.

Marcovich (287) has made an attempt to determine the nature of the active group in the acridines which is responsible for the ability of these sub-

stances to induce the cytoplasmic loss leading to the "petite" condition. Carbon 5 appears to be the key position since substitutions on it lead to loss of "mutagenic" activity. It is of interest to note that in addition to the acridines certain triphenylmethane dyes were found to be active. Marcovich notes that the angle subtended by the middle carbon of the latter compounds is the same as that of carbon 5 in the acridines.

Slonimski (389) has observed a close correlation between the ability of a compound to induce the "petite" phenotype and its ability to inhibit the formation of the respiratory enzymes in the wild type. He is able to demonstrate that the inhibition of enzyme formation precedes the "mutagenic" effect.

Campbell & Spiegelman (84) have employed "petite" mutants of *S. chevalieri* in an attempt to decide whether the postulated cytoplasmic enzyme forming elements are identical to functionally active enzyme molecules. Samples of mass reverting populations were assayed on a cellular level for enzyme content by plating on pure glactose and for capacity to form galactozymase by plating on galactose plus another energy source. Since enzymatic activity was found to disappear much faster than the capacity to form more enzyme, it was concluded that the enzyme forming system is not identical to functional enzyme in these strains. It has been found possible to convert large fractions of "long term adapting" cultures to the galactose positive state (366). The fact that active transforming preparations can be obtained from both galactose adapted and unadapted cells leads the author to the conclusion that a preexisting but inactive enzyme-forming system is being activated.

In the last year one paper from the Lindegren laboratory claims that the Winge & Roberts phenomenon ("long term adaptation") in *S. chevalieri* is attributable to mutation and selection (272), and another by the same authors states that it is not (332). From their study of adaptation to galactose in *S. cerevisiae* Lindegren & Pitman (272) conclude that "galactose by collision with the gene converts it to the positive state." Certain technical complexities (e.g., the occurrence of from 3×10^6 to 3×10^9 divisions on their test plates in fluctuation tests) make one hesitate to accept the above conclusion.

Rizet (358) has described a unique cytoplasmic phenomenon in the ascomycete *Podospora anserina*. In this organism, two allelic genes S and s determine a certain growth pattern ("barrage") when mycelia of each type are juxtaposed on an agar plate. Of the progeny of S s crosses, 50 per cent have the S character. The remainder, called s^s, give rise to mycelia which are genotypically s but phenotypically resemble the S character. These S-phenocopies can be propagated indefinitely in the vegetative condition. However, contact with an s mycelium infectively transforms an s^s mycelium into the s condition. Crosses of s^s microconidia to s mycelium give only s progeny, whereas the reciprocal cross (301, 358), s to s^s, yields only s^s progeny Rizet has recently shown (360) the existence of three other genes concerned

with this phenomenon and has demonstrated infective transmission of the barrage character amongst strains carrying each of them. Incompatibility in crosses of different *Podospora* isolates is related to the barrage phenomenon but it is also affected by other genetic factors (361). Again in *P. anserina*, Rizet (359) has uncovered a situation analogous to the necessity for nuclear reorganization in paramecia. He finds that all the strains of *Podospora* in his possession eventually stop growing and die if propagated vegetatively. The plus mating type survives longer than the minus mating type. Retention of the strains requires re-isolation from ascospores.

Nucleo-cytoplasmic interrelations.—The effect of enucleation on synthetic processes in the alga *Acetabularia* has been studied by several investigators. It was found that the ability of the plants to form gametangia support structures ("hats") is not abolished by enucleation (60). Indeed, in young plants "hat" formation occurs much faster in the enucleated fragments than in the nucleated controls. It is concluded (61) that the presence of a growing nucleus inhibits hat formation. In another investigation (407) of the same organism, it has been demonstrated that the ability to form metaphosphate is not dependent upon the nucleus.

Brachet & Szafarz (70) have studied the incorporation of labeled orotic acid into the RNA of nucleated and enucleated halves of *Acetabularia*. They demonstrate active incorporation in the enucleated halves and the maintenance of high incorporation activity over a considerable period (71 days). Their experiments definitely establish that the presence of any of the nuclear components is not necessary for the active synthesis of RNA.

Nanney (310) has proposed a theory for mating type determination in *Paramecium aurelia* which depends upon the ploidy of the macronuclear subunits. In its essence, this theory seeks to explain inheritance of mating type via a cytoplasmic substance produced under the influence of the macronucleus. This theory was subjected to critical test by means of parthenogenetic matings involving the passage of only one nucleus between the mating pair (Sonneborn, personal communication). The results demonstrate that mating type is determined by the origin of the entering micronucleus and not by the resident macronucleus.

Nanney (311) has analyzed the process of conjugation in *Tetrahymena*, a form which has both macro- and micro-nuclei. The process, although differing in details from that observed in *Paramecium*, is quite similar in its broad outlines. The results obtained indicate that the fate of the nuclei resulting from the meiotic divisions is controlled by a complex series of nucleo-cytoplasmic interactions analogous to those demonstrated in *Paramecium*.

Persistent variations.—New examples of the effectiveness of anti-sera and temperature variations in directing lasting alterations in the antigenic structure of bacteria have been reported. Bruner (77) employs monotypic sera to induce persistent change in the somatic antigens of the genus *Salmonella*. Temperature lability of antigenic components in *E. coli* has been ob-

served, and evidence has been obtained which links sensitivity to different phages to the presence or absence of the temperature-sensitive antigens (317). Beale (44) summarizes investigations along similar lines which have been carried out with *Paramecium* and also discusses analogous phenomena concerned with resistance to various lethal agents.

Wright has uncovered an instance of phenotypic variation in the course of studying the reversion of a glycineless mutant of *E. coli*. It was first noted (461) that the presence of certain α-hydroxy acids (e.g., lactic, glyceric, tartaric acids, etc.) increased the frequency with which partially reverted cells appeared. Growth of the partial revertants on minimal was characterized by a stepwise increase in growth rate (463). Plating on minimal medium from cultures showing this phenomenon revealed the existence of two cell types, one corresponding to a fast grower and the other to a slow. Presumably the dissimilarity between these two is referable to differences in glycine-synthesizing capacity. The data on the numbers of each type present indicate that there is a mass conversion from one to the other, rather than a selection for the fast type. The inclusion of glycine in the minimal medium virtually abolishes the conversion (462, 463).

This phenomenon is termed "auto-adaptation." Formally, it is equivalent to an extended phenomic delay in which the acquisition of the glycine synthetic capacity is discontinuous in the population, occurring earlier in some cells than in others. The ability of glycine to prevent the conversion from slow to fast synthesizers may be related to the phenomenon described by Monod and collaborators (102, 103, 299, 300) in which suppression of enzyme synthesis is attained by adding the homologous product metabolite.

Interest has been revived in the neglected observations of Harder (179a), who was one of the pioneers in the experimental analysis of cytoplasmic autonomy. Harder took advantage of the clamp-connector mechanism of division in the basidiomycetes to obtain monocaryotic mycelia with dicaryon-derived cytoplasm. Aschan (21) and Fries & Aschan (154) used similar material and the micro-surgical technique of Harder. In addition they took advantage of an organism, *Collybia velutipes*, which undergoes spontaneous dediploidisation to form monokaryotic oidia. Examination of the "neohaplonts" formed by either the spontaneous or micro-surgical procedures yielded strains with persistent differences, though from the mode of their origin, they should have been of like genetic constitution. Aschan & Norkrans (22) examined the same organism for ability to produce a cellulose splitting enzyme. "Neohaplonts" of a given genotype showed appreciable variations in enzyme synthesizing capacities. The authors point out that nuclear differences may have arisen in their material via mutations and to this extent provide an alternative explanation for the phenotypic variations observed.

Many aspects of the great variability of color in strains of *S. marcesens* are highly suggestive of a cytoplasmic mechanism (249). Further analysis will however be required in order to identify the nature of the processes operating here.

Luria (276) has recently reviewed his own contributions and those of others to the study of host-induced modifications in the bacteriophages. As an example of this phenomenon we may cite one of the cases studied by Bertani & Weigle (59, 452). Coliphage λ, which is normally lysogenic in K-12, infects and grows in strain S of *E. coli*. The progeny produced are indistinguishable from the original λ. If, however, λ is grown on strain C of *E. coli*, viruses are produced (designated by λ-C) which plate with normal efficiency on C but with very low efficiency on strain S. One passage through S converts λ-C to λ. Mutation-selection has been eliminated as a possible mechanism in the explanation of this phenomenon. Similar observations have been made involving phages P2, T2 and T6 and strains of *E. coli* and *Shigella* (59, 276, 452). Further, the change in plating specificities observed on passage of Vi-typing phages through "degraded" strains of *Salmonella typhi* has recently been recognized as resulting from host-induced modifications (10).

Temporary variations.—Phenotypic variants in populations of bacterial viruses involving resistance to heat inactivation (2), to chelating agents (247a), and to osmotic shock (13) have also been described. The osmotic-shock-resistant proportion of the phage population can be altered at will by adjustments in temperature.

Any heterogeneity in the distribution of an enzyme-forming capacity in a population of cells constitutes an example of variation which can be exhibited by placing the cells on media containing the inducing substrate as a sole carbon source. Only those cells competent to respond by synthesizing enzyme will produce visible clones. Ravin (356) studied such a situation in *Aerobacter aerogenes* when utilizing various Krebs cycle intermediates. That enzyme synthesis is a prerequisite to the utilization of such intermediates is strongly suggested by recent investigations of Kallio and his co-workers (40). Norman (318) has exaggerated the same phenomenon by the use of ultraviolet irradiation which is known to inhibit enzyme synthesis and survival differentially. Thus survival rates of irradiated glucose-grown cells of *Aerobacter* are strikingly lower on lactose than on glucose. If, however, the necessity for initiating the synthesis of a new enzyme is removed by prior adaptation to lactose, the survival curves on glucose and lactose are found to be identical. It is of interest to note that Norman found that the survival curves of adapted and nonadapted cultures plated on lactose extrapolated back to the same point. The data obtained on lactose are paralleled by results obtained with acetate, citrate, succinate, and rhamnose. Similar effects of the medium used on survival with and without radiation have been observed by Stein & Harm (180, 398). These authors discovered that incubation of seeded plates at elevated (44.5°C.) and low (10°C.) temperatures prevents the apparent inactivating effect of the medium.

An interesting but rather complicated situation was observed by Berger & Wyss (53) in the case of resistance of *M. pyogenes* to phenol. Resistant mutants could be selected relatively readily but apparently rapidly lost the phenol-resistance phenotype on growth in the absence of phenol. The be-

havior could be explained if the phenol-resistant phenotype were an inducible state.

Eddy & Hinshelwood (132) have noted the existence of a small number of cells in populations of *B. lactis aerogenes* which show a much greater resistance to the lethal action of an acridine and other injurious agents than the bulk of the population. They demonstrated by subculture that these survivors are not heritably resistant. A similar phenomenon was observed by Markert (288), who noted heterogeneity with respect to ultraviolet-radiation-resistance in *Glomerella*. A small number of resistant conidia accounted for the plateau in the survival curve at high doses of ultraviolet light. It was found that the resistant conidia can be distinguished by the extent of their melanin pigmentation. Their resistance is very likely a result of the protective effect of the pigment. The coloration and the resistance are both a function of age and are not transmitted. Analogous, though unanalyzed, inhomogeneities were observed in experiments with yeast (375, 460).

Another instance of phenotypic variation in resistance is illustrated by the very marked variation in sensitivity to streptomycin in cells taken from different portions of the growth curve (69). A temporary change in the mating phenotype of *E. coli* (F^+ behaves as F^-) (189) may be brought about by aeration while anaerobiosis, high temperatures, and various chemicals shift the response of bacterial populations to subsequent lysogenic phage infection (58, 280).

Gene Action

From its inception microbial genetics has held out the hope of providing information relevant to the problem of gene function in terms of mechanisms interpretable at the enzymatic and biochemical level.

The survey of papers in the preceding section has demonstrated the wide range in autonomy of the systems determining the expression of phenotype. At one end of the scale we have such elements as kappa and plastids; at the other, the evanescent variation of resistance to streptomycin which characterizes the different growth phases of *E. coli* (69).

It is therefore not surprising to find that clues concerning the mechanism of gene action drawn from the multitudinous microbial researches should yield suggestions which are not always in agreement with each other. Nevertheless, as experimental facts they must ultimately find explanation in a unified theory of gene function. Our major purpose here is to summarize the pertinent information which has accumulated in the past year.

Enzymatic constitution and gene action.—A fairly straightforward relationship between genes and enzymes is implicit in the finding that gene mutation to a biochemically more exacting state results in the loss of the pertinent enzyme activity. Several such cases have been described (118, 370, 385, 414, 436). Partial, quantitative loss of enzyme may lead to partial requirements. This is elegantly demonstrated in a series of mutants partly or completely blocked in the synthesis of 5-dehydroshikimic acid, a precursor for several aromatic metabolites. Complete loss of enzyme leads to a requirement for

tyrosine, phenylalanine, tryptophan, PABA[2] and POB[2]. If one per cent of the normal enzyme activity is present, only tyrosine and phenylalanine are required (109). Quantitative changes in enzymes due to gene mutation are described by several authors (208, 379, 385, 414).

A qualitative, structural, change in an enzyme which is associated with a single gene mutation has been described by Horowitz & Fling, who have isolated a heat-labile tyrosinase from a mutant of *Neurospora* (208). The authors discuss the view that structural change in enzyme in response to gene change may indicate the possibility of a fairly direct control of genes over enzymes.

Synthesis of new enzymes in different types of mutants has been reported. The appearance of rare penicillinase-producing strains of *Micrococcus pyogenes* (418) and of chloromycetin-reducing strains of *E. coli* (292) has already been noted. A new cytochrome band is found in a mendelian oxidative mutant of *Neurospora* (295), and a new enzyme activity, cytochromase, is found in the analogous cytoplasmic "mutant" (185). This latter case is of a very special interest, since gene mutation is apparently not involved. Similar interest attaches to an observation of Cohen (99) that a thymineless mutant of *E. coli* becomes capable of thymine synthesis after infection with T2 bacteriophage. In all the above instances the presence of trace quantities of enzyme in the wild type is not easy to eliminate. Finally, Fredericq (150) describes a single mutational event in *E. coli* which results in resistance to phages T5, T1, and a colicine, and the simultaneous acquisition of the ability to produce a new colicine, M.

Two cases of pleiotrophic mutation have been reported by Sussman & Markert (414). Other cases of pleiotrophism have already been noted in discussing effects associated with acquisition of resistance to both phages and antibiotics.

Gene action and induced biosynthesis of enzymes.—Understanding of the mechanism of enzyme synthesis is an obvious prerequisite, or at least corequisite, to the attainment of an adequate model for gene function. Important advances have been made in this area, previously referred to as "enzymatic adaptation" (104). Benzer (49) has employed a most ingenious technique to establish that, under certain conditions, enzyme synthesis proceeds uniformly and at comparable rates in the vast majority of the population of cells being induced. Deductions concerning the kinetics of enzyme synthesis can, under such circumstances, be made with a greater certainty than previously attainable. Pollock & Torriani (333, 334) provide convincing evidence that the inducer functions catalytically, rather than stoichiometrically, in the synthesis of penicillinase by *B. cereus*.

Evidence continues to accumulate (177, 178, 394) emphasizing the importance of free amino acids as precursors in enzyme synthesis. Definitive data on this question has been provided by Cohn & Hogness using S^{35} (cited in Monod & Cohn, 300a) and Rotman & Spiegelman (367) using C^{14}. Both groups of workers arrive at the conclusion that the synthesis of

β-galactosidase in *E. coli* is a *de novo* process involving neither simple nor complex pre-existent precursors, a possibility suggested by previous evidence (105). Such data provide encouragement to those who postulate a template mechanism, capable of synthesizing enzyme molecules directly from their component amino acids (208).

On the other hand, complexities of genic control over enzyme synthesis which challenge the adequacy of any simple view of gene-enzyme relations have also emerged from studies of induced enzyme formation. Lindegren and his collaborators (266, 324) describe a series of alleles which control the synthesis of an α-glucosidase apparently capable of hydrolyzing six different oligosaccharides. The evidence offered that only one enzyme is involved is as yet incomplete. The alleles are differentiated from one another on the basis of their response to various inducers. In the course of these studies the authors confirmed earlier observations that substrates need not necessarily be effective inducers for their homologous enzymes.

Cohen-Bazire & Jolit (101) describe a mutation in *E. coli* which causes the normally inducible enzyme β-galactosidase to be formed constitutively. Enzyme formation in such constitutive mutants is inhibited by substances which stimulate this process in inducible strains. Inhibition of enzyme synthesis by the normal enzyme substrate has been noted in several cases (102, 103, 299, 300), and has been attributed to interference between these substrates and hypothetical endogeneous inducers (103). Study of enzymes along a pathway interrupted by gene mutation has permitted Vogel (435) to demonstrate the functionally inducible nature of the synthesis of an enzyme which normally appears as constitutive.

Modifier and suppressor action.—A variety of modifier effects have been reported during the past year. Pantothenic acid requirement in a *Neurospora* strain is dependent on the ionic composition and the biotin content of the medium, but these effects are eliminated by crossing (373).

Evidence for a plausible biochemical explanation of a supressor effect concerned with the pyruvic decarboxylase reaction has been obtained by Strauss(412, 413), who has shown that an inhibitory metabolite accumulated as a result of the first mutation may be reduced as a result of the suppressor mutation. Adelberg & Umbarger (3a) have observed that the suppression of the valine requirement of an isoleucine-valine mutant of *E. coli* is correlated with a substantial increase in one of the two valine transaminases. The increased activity presumably permits adequate valine synthesis, while the absence of the other transaminase still prevents isoleucine formation. It has been shown that gene mutation may relegate metabolically active enzyme to a cryptic state (101), or it may isolate an enzyme forming capacity in a metabolically unusable pathway (98). A possible instance of hybrid vigor in microorganisms has been reported (268).

Newmeyer and Tatum (316) describe four different genes which modify the expression of a tryptophan-nicotinic acid mutant of *Neurospora*, by shifting the apparent location of the metabolic lesion. A hypothesis in terms of

intermediate metabolism which could account for these results is given by the authors. In extension of previous work, Yanofsky (470) has obtained 23 tryptophanless mutants of *Neurospora* which lack tryptophan desmolase and presumably are allelic. Three different suppressors which partially restore this enzyme have been found. Each of them suppressed only one or two among the different alleles tested. Analogous results involving one suppressor and three to eight alleles at an inositol locus of *Neurospora* have been reported by Giles & Partridge (162).

While some of the foregoing results point toward a high degree of gene interaction, a remarkable instance of gene autonomy has been plausibly postulated by Stocker, Zinder & Lederberg (411). In transduction experiments with *Salmonella*, a gene for motility [i.e., flagella synthesis (H)] is introduced into a nonmotile cell of the receptor strain. Once there, it fails to displace its inactive allele, but it nevertheless confers motility on the receptor cell. This cell continues to throw off nonmotile daughter cells along its path of travel. If it can be confirmed that the H gene fails to duplicate, a complete separation of hetero- and autocatalytic gene functions will have been established here.

Multiple factors and pseudoallelism.—Quite a few instances of multigenic control of individual traits have been reported or analyzed further. These include: "barrage" in *Podespora anserina* (360); raffinose and maltose fermentation in yeast (220, 456); production of α-glucosidases in yeast (271); N-mustard, penicillin, chloromycetin, and furadroxyl resistance in *E. coli* (91), and motility and flagellar synthesis in *Salmonella* (411).

Information relevant to the size and stability of the primary gene product can presumably be gained from a study of the interactions of pseudoallelic mutants in heterocaryons and in diploid nuclei (where the mutations can be respectively in repulsion or coupling). In this connection it is of interest that numerous pseudoalleles are found to yield phenotypically wild strains in the disomic state (331), while the same strains generally appear to form nutritionally-exacting heterocaryons.

Gene action theory.—One of the most serious objections to the "one gene-one enzyme" hypothesis stems from the suggestion that the biochemical mutants which furnished the evidence in support of the hypothesis were atypical of gene mutation in general. According to this concept it was conceivable that the mutants unrecoverable by the nutritional supplements usually provided might well constitute a large majority of the mutable genome and that these undetected genes could well be multifunctional. Leupold & Horowitz (256) performed experiments to cope with these objections. They collected 161 temperature mutants from ultraviolet-treated cultures of *E. coli* by isolating those strains which grew at 25°C., but would not grow at 40°C. These strains were then classified on complete medium at 40°C. Of the total, 124 possessed reparable deficiencies, and 37 were irreparable by external supplements. Making the assumption that temperature mutation is as likely for irreparable functions as for reparable ones, these authors concluded that most gene functions are reparable.

Using a different experimental system, Atwood & Mukai (23) have arrived at the opposite conclusion. These workers collected 58 spontaneous mutants by an ingenious technique (22a) based on the survival of deficient nuclei in heterocaryons. Tests for allelism were carried out amongst these mutants, and 26 of them proved to be distinct. Of the latter, two had reparable functions, while 24 were irreparable in complete medium. Atwood & Mukai believe that the discrepancy between their results and those of Leupold & Horowitz can be explained if temperature mutations for irreparable functions are less probable than for reparable ones. The heterocaryon method would appear to be a superior method for the preservation of "lethals" than that available to Leupold & Horowitz.

TRANSMISSION GENETICS AND LIFE CYCLES

The present section summarizes recent information relevant to the mechanisms and modes of gene transmission, and by extension of this topic, to life cycles. These subjects stand in contrast to the preceding discussion which dealt with gene function and to the subsequent section which is concerned with the properties of genes and genetic material.

Virulent Viruses

The investigations in the bacterial viruses constitute one of the largest components of the published material relevant to the subject of microbial genetics. One textbook (275) and the proceedings of three symposia (140a, 278a, 441a) have appeared in the last year containing descriptions of various aspects of viral genetics. Only the proceedings of the two most recent symposia, held respectively at Royaumont (France) and Cold Spring Harbor (New York) are reviewed in detail.

The viruses are the only organisms whose life cycle is being analyzed on a chemical level in investigations which combine many of the techniques of modern immunochemistry and tracer chemistry. We have included much of this information because we regard it as an integral part of the knowledge necessary to an understanding of the genetics of the viruses.

Taxonomy and morphology.—Adams (1) discusses the possibility of a biological classification of the bacteriophages based on their serological, morphological, and physiological properties.

Some new details concerning the morphology of T phages have been described: Lanni & Lanni (247) have shown that T2 possesses two antigens, one associated with the protein coat and "doughnut" (84) and another, homologous to phage neutralizing antibodies, associated with virus tails. Anderson and his collaborators (12, 13) have further delineated the osmotic properties of T4 and T6 and have described structural details of these phages. Lark & Adams (247a) have obtained evidence for the presence of metal ions in the structure of phage T5 which exert a stabilizing influence. The DNA liberated from "surface-tension disintegrated" phages is in the form of fibers of diameter 20 ± 5 Å (149). The cytological events in phage infections have

been studied with both the light microscope (308) and the electron microscope (304).

Invasion.—Many informative details concerning the mechanisms and factors involved in phage invasion have been reported. In a review of his own work, Beumer (62) has described the isolation from bacteria of various receptors which inhibit those phages to which the bacteria are susceptible. These receptors can adsorb antibacterial antibodies which inhibit phage adsorption and are found only in susceptible strains. Weidel (449, 450) has shown that receptors for a given phage are not found in the lysate which results from its invasion, although receptors for other phages can be detected. He has studied these receptors chemically and proposes a tentative receptor-network structure for the surface of *E. coli* B on the basis of his data. "Semi-resistant" mutants of *E. coli* fail to adsorb phage (439).

Nicolle *et al.* (317) have shown that the antigenic structure of an *E. coli* strain changes drastically with the temperature and that these changes are accompanied by changes in susceptibility to several phages. Jesaitis & Goebel (163, 223, 224) have isolated a pure lipomucoprotein antigen from *Shigella sonnei* which inactivates T2, T6, T3, T4, and T7 *in vitro*. A mutant resistant to T3, T4, and T7 produces an analogous fraction which only inactivates T2 and T6. They found that 2×10^{-16} gm. of lipocarbohydrate derived from the wild type causes instantaneous release of DNA from a T4 particle (which weighs roughly 10^{-15} gm.). Summing up recent and older information in a review, Puck (350) describes the first step in virus invasion as consisting in the formation of an electrostatic bond between virus and a specific site at the cell surface. Under special conditions, however, infection of a few cells may be forced even in the absence of specific receptors (448). Further, phage may be adsorbed on ion exchange resins *in vitro*. This relatively nonspecific adsorption is sometimes followed by release of the DNA from the phage coat (350a).

Origin of phage DNA.—The origin of bacteriophage DNA has been the subject of several review papers (139, 166, 192, 239) and of renewed experimental investigation. Study of the transfer of parental DNA to progeny phage has confirmed that no more than 50 per cent of the input radioactivity is passed on, most of it to the first few finished phages (194, 447). Evidence from experiments with T2 heavily labeled with P^{32} indicates that 12 out of 13 of the P atoms in the DNA of this phage can suffer conversion to S^{32} without impairing its reproductive capacity (401). Depending on the phage strain under study, from 15 per cent to 70 per cent of its DNA-phosphorus has been assimilated by the bacterium after infection (139) (postassimilated). In T2 and T4, about 30 per cent of the phage DNA is preassimilated, and about 70 per cent postassimilated (194, 403). The preassimilated phage DNA is probably all derived from the bacterial DNA. There seems to be slight disagreement on the rate at which the host DNA becomes available for phage synthesis (194, 195, 403). All of the bacterial DNA appears to be potential raw material for phage synthesis (195). The flow of

P^{32} from the bacterial DNA and the medium to a pool of noninfective phage DNA has been studied in T2 by Hershey (194, 195). Such a pool can be identified because its DNA contains phage-specific 5-hydroxymethylcytosine and is sensitive to desoxyribonuclease. The phage precursor pool builds up to the equivalent of the DNA content of 50 to 80 phage and then remains roughly constant as infective particles begin to appear (194, 195).

The average period required for transport of P^{32} from the medium into the pool is estimated at from 7 to 9 min. and from pool to phage at from 7 to 8 min. (194, 403). Reactions leading into and out of the pool appear to be irreversible. If a superinfection is forced by very high multiplicities 6 to 8 min. after the first infection (432), the superinfecting markers enter into the phage pool immediately and may be recovered in recombinants as early as 4 min. after the infection (434). Although their genetic markers are not represented, from 20 to 30 per cent of the P^{32} of superinfecting unrelated phage (mutual exclusion situation) also appear in the progeny (447). On the other hand, much of the P^{32} of certain strains of superinfecting phages may be released into the medium by a special, and an as yet undefined mechanism (165).

The ultraviolet-radiation-resistance method of study of intrabacterial phage development originated by Luria and Latarjet has been extended to several phage-bacterium systems by Benzer & Jacob (50). Some systems, such as *E. coli* B and phage T7 give seemingly straightforward results, but others, such as *E. coli* B and phage T2, require complex interpretations.

Origin of phage protein.—The synthesis and origin of phage protein can now be regarded as a problem distinct from that of phage DNA. In his best experiments, Hershey found as little as 0.5 per cent of the parental phage S^{35} in the progeny (193). Two groups of researchers found evidence for the independent synthesis of the phage protein coat in the bacterial cell (115, 259, 281). "Doughnuts" (115, 259), which are believed to correspond to the protein coat of the phage, are recovered from phage-infected bacteria grown in the presence of proflavine or from bacteria which have been broken open early in phage development. They contain 75 per cent of the total phage sulfur and less than 15 per cent of its phosphorus and are not adsorbed on bacteria. "S^{35} containing particles" (281) obtained from KCN-induced or mature lysates are however adsorbed on sensitive bacteria. In analogy to the phage DNA pool, a pool of S^{35} particles accumulates prior to the appearance of infective phage. The S^{35} label takes however only an average of from 6 to 7 min. to travel from medium to finished particle.

Host metabolism.—The effects of phage infection on the host cell have been investigated in several laboratories. An increased phosphorus requirement is reported in infections of *E. coli* with the T-even phages (309). Cohen (99) reports the loss of a thymine requirement in T2 infection of a thymineless mutant of *E. coli*. Enzymes concerned with purine and pyrimidine metabolism have been followed in T4r-infected and normal *E. coli* (152). An increase in desoxyribonuclease activity has been found in infected cells (326),

and has been shown to result from the destruction of an inhibitor (239). Cohen and collaborators (97, 99, 100) have shown that phage infection may force a shift from the phosphogluconate pathway of glucose utilization (leading to riboside synthesis) to the glycolytic pathway (leading to desoxyriboside synthesis). The mechanism of this shift does not involve loss of phosphogluconate pathway enzymes (99, 100), but might be attributable to inhibitions caused by the nonbacterial metabolite 5-hydroxymethyl cytosine (99). Correlations between cell size, RNA, DNA, and protein content, and phage yield have been obtained (320).

New experimental systems.—Work on new bacteria-phage systems has been initiated (151, 442). Peculiar variations in plaque morphology and host-range behavior have been observed by Raettig (352), but the methods employed by this author make it impossible to evaluate the significance of his findings.

Recombination in phages.—The papers on genetics of bacteriophage published during the past year have been dominated by the theory of recombination proposed by Visconti & Delbrück (433).

This theory holds that several rounds of mating occur, on the average five per virus particle, and that most recombination takes place after a good deal of multiplication has taken place. Some of the experimental results obtained in the past, reviewed by Hershey (191), are explained admirably by the theory. Among these may be mentioned the occurrence of recombinants incorporating markers from three different parent phage and the preponderance of recombinants over the minority parent type in systems where recombination is known to occur late in the duplication process. Furthermore, certain predictions of the theory have already been confirmed by analysis of new and published data. For example, a drift toward genetic equilibrium is to be expected as random mating proceeds, and has been found to occur (122, 260, 431). The theory also predicts that phages which have participated in at least one mating, recognizable as recombinants, are more likely to show recombination between other unselected markers than are randomly selected progeny. Formally, this situation is equivalent to negative interference. It made its appearance in some of the older experiments (433) as well as the more recent ones (122, 123). *B. megatherium* 899 A and phage T, a phage-bacterium system recently developed for genetic study (306a, 307), also appear to show this phenomenon, although the linkage in the five-membered linkage group observed is anomalous in being extremely close (307). Further experimental analysis seems desirable before these organisms are used as a test system. Recombinant types incorporating markers from three different parents have been obtained by Bresch (72) in phage T1 marked with plaque-mutants and scored on dyed agar.

Some data remain which are not explained in any obvious manner by the Visconti-Delbrück hypothesis. Notable among these are the phage heterozygotes (122, 191). Levinthal (258) has shown that the structure of the heterozygotes corresponds to overlapping strands of genetic material rather

than to inclusions of duplicate fragments of strands. Their origin remains obscure. Recombination results which do not fit any current theory are reported for T3 (147).

Stent (402) stored T2hr marked heavily with P^{32} at −196°C. and observed the decay of individual loci in the subsequent crossing experiments with $T2h^+r^+$. The data on the recovery of the four genetic classes suggest that the h and r loci duplicate independently as indicated in previous experiments with ultraviolet and x-ray inactivated phages.

Recombination in animal viruses.—Burnet and his collaborators have recovered parental strains from crosses of previously obtained recombinants of influenza virus (265). The occurrence of recombination has been further confirmed by newer methods (78, 203), and evidence for a noninfective unstable combinant state which might correspond to heterozygosity has been uncovered by both Burnet's and Hirst's groups (79, 202, 204). Experiments with ultraviolet-inactivated viruses have been initiated (14) in an attempt to demonstrate multiplicity reactivation.

Lysogeny

"Lysogeny occupies a privileged position at the crossroads of normal and pathological heredity, of genes and viruses" (Lwoff, 278).

Previous reviewers could justify the omission of lysogeny as being beyond the scope of a review of microbial genetics. Today, however, an inclusive discussion of bacterial genetics can scarcely be essayed without it.

A detailed and brilliant review of the present state and historical development of the subject of lysogeny by Lwoff (278), replete with erudite quotations, has appeared late in 1953. The terminology employed in this rapidly developing field is defined here and elsewhere (215).

Induction.—Induction of phage development by physical, chemical, and biological agents is reviewed separately by Lwoff (279) and by Jacob & Wollman (217), and specifically for *M. pyogenes aureus* by Welsch *et al.* (454). The ability of a virulent mutant to induce development of its temperate counterpart is reported, and the interaction of the two phages in multiplicity reactivation is described (217, 218). Jacob (213) has studied the induction of phage development in a polylysogenic strain of *Pseudomonas pyocyanea* for related and unrelated phages. Spontaneous development of two related phages simultaneously is found to occur with a frequency greatly exceeding random coincidence, but ultraviolet induction of the two phages is random. On the other hand, in *Shigella* doubly lysogenic for phages P1 and P2, Bertani (55, 56) finds either one or the other phage in a single burst but not both. Herzberg & May (196) describe a lysogenic strain of *E. coli* which yields a single phage in cultures of from 50,000 to 100,000 bacteria. Details of the induction process are reported for a *Salmonella typhimurium* phage (280), and the effect of various chemicals, temperatures, and pH values on induction in *Salmonella* are described (391). On the basis of many detailed results, the conclusion has been reached that inductibility depends primarily on the

properties of the phage, and secondarily on the physiological state of the bacterium and on extrinsic factors (217, 279).

"Decision," preprophage and prophage states.—Several papers have appeared which deal with the "decision" in phage infection of a bacteria to (*a*) be lysogenized (reductive infection), (*b*) be lysed (productive infection); (*c*) be killed; (*d*) remain sensitive. Different phage-bacterium systems behave differently under a given set of conditions. High multiplicity of infection leads to prevalence of (*a*) in *S. typhimurium* infection with phage A (280) but not in *E. coli* K12 S infection with λ (263). In the K12S-λ system, decision between (*a*) and (*b*) is unaffected by temperature (263), while this is an important factor in the lysogenization of *Shigella dysenteriae* (55, 56, 58) and *Salmonella typhimurium* (280).

Interesting hints concerning a preprophage state intervening between cell invasion and the establishment of the prophage condition by the infecting phage is adduced by several authors. Lieb has shown that the preprophage of λ is more temperature-sensitive than its free phage or prophage forms (262). A mutant of λ, L-vl-t, may be carried for 12 cell generations, presumably in the cytoplasm of strain K-12, before the decision between reductive and productive infection occurs (263). Bertani finds (56) that the decision can be influenced as late as 20 min. after infection by varying temperature conditions.

Evidence for an intermediate state between prophage and vegetative phage has also been uncovered. Thus, addition of 5-CH_3-tryptophan during the first 6 min. after induction can restore the lysogenic state almost completely in *S. typhimurium* (280). Ultraviolet-induced *Pseudomonas pyocyanea* which have a latent period of 85 min., can be "photorestored" [i.e., induction can be reversed by illumination, 20 min. after the induction (217)]. However, photoreversibility is not observed in x-ray or N-mustard induction.

Genetic interactions between phages and between phage and host.—Interactions between sensitive or singly and doubly lysogenic cells and one or more infecting phage are discussed by Bertani (55, 56, 57), Jacob (213), Jacob & Wollman (217), and Lwoff *et al.* (280). All workers in the field report inability to obtain triple lysogenicity for closely related phages, whereas double lysogenicity can generally be obtained (55, 56, 263). This is taken to mean that only two cellular sites are available for a given phage and its derived strains. Further support for this view is found in the fact that a prophage is sometimes replaced by a related superinfecting phage (55, 56). Impressive evidence that these sites are located on the bacterial chromosome is presented by Lederberg & Lederberg (250), Wollman (459), and Appleyard (15). All are in agreement that crosses of lysogenic to sensitive *E. coli* K-12 yield both parental types. Sensitive segregants have also been obtained in crosses between sensitives and lysogenic heterozygotes (250). These results, and particularly those which establish linkage between lysogenicity and the fermentative marker Gal_4 (250, 459), virtually rule out a cytoplasmic transmission of the prophage. Lederberg & Lederberg (250) recognize one locus with three alleles controlling lysogenicity in K-12 and another locus with two

alleles controlling phage adsorption. They also note the existence of modifiers. Prophage does not, however, behave as an ordinary gene in all respects. The presence of, or absence of, the lysogenic alleles has a marked effect on the transfer of the Gal locus (15). Recombination between markers in related lysogenic phages may occur when both phages are in the prophage condition (15), or prior to multiplication when both phages are in the vegetative condition (217). More commonly, it occurs during the multiplication phase of phage development (217, 218).

The physiology of lysogenic phage development.—The nutritional requirements of temperate phage development and the changes attendant upon their development in the bacteria form the subject of several papers (164, 211, 214, 319, 386). Thus, the ionic requirements of phage growth are shown to be more exacting than those of bacterial growth in the case of a *B. megatherium* phage (211). Likewise, numerous amino acids which are necessary for λ production by K-12, are not necessary for bacterial growth (164). On the other hand, bacterial growth requires more than ten times more glucose and much more nitrogen than the liberation of 50 temperate or virulent phages. Bacterial generation time is much more severely affected by carbon starvation than phage production time or yield (214). Metabolic changes in bacteria subsequent to induction of temperate phage development are compared to those observed in virulent phage infection and found to differ on numerous counts (217, 386).

Lysogenicity-associated characters.—As a result of the acquisition of the lysogenic character, some associated properties are found in bacteria which may be of very great theoretical and practical importance. Groman (172, 173, 174) and Barksdale & Pappenheimer (36) have confirmed Freeman's discovery which revealed that toxin production in *Corynebacterium diphtheriae* is acquired together with lysogenicity. The same subject is also discussed by Hewitt (197, 198) who does not find complete correlation between these characters. Spores of a strain of *B. cereus* pathogenic for insects, were found to contain crystals reminiscent of polyhedral inclusion bodies. No crystals were found in nonpathogenic strains (179). The acquisition of a different colony morphology with lysogenicity has been mentioned (212). Lederberg & Lederberg (250) report that all λ-sensitive strains of *E. coli* obtained from a large natural collection were found to be fertile with K-12. Finally, a direct consequence of lysogenicity, immunity to superinfection by the acquired phage, is utilized in phage typing of *Salmonella* strains. These relationships have only been recognized recently and much of the detail has been worked out (9, 10).

Bacteriocins.—The similarities and differences between bacteriocins such as colicin and pyocin and the homologous phages form the subject of two review papers (150, 216). Analogies extend to induction mechanisms of phage and bacteriocins, genetic control, mode of action, host range of action and production, metabolic changes in the bacteria due to synthesis of the agents, behavior and occurrence of mutants resistant to agents. The evidence is highly suggestive that bacteriocins correspond to phage protein coats (216).

Bacteria

Bacterial life cycles.—Much still remains to be done in the elucidation of the life cycle of bacteria and the connection of the L-forms and other morphological variations with this process. So far, these connections are sustained almost exclusively by cytological observations. The origin of L-forms from normal bacterial cultures and the recovery of normal cells from L-forms has been described for streptococci (120), *Proteus* (438), corynebacteria (293), and *Azotobacter* (125). L-forms have been obtained in *E. coli* by growing this organism under quasi anaerobic conditions (210). Time sequence observations have suggested the possibility that L-form formation may be genetically determined, that it does not involve cell fusion (125), and that it may be a result of a form of endomitosis (438). The wide range of forms which appear sequentially and simultaneously in a given culture is described (121, 125, 438). The possibility that morphological variability may sometimes result from experimental artefacts is suggested by data obtained with pleuropneumonia organisms by Liebermeister (264). This author found that direct transfer of cultures from agar to collodion membranes, followed by very careful fixation, yielded preparations consisting purely of tiny spherical bodies of uniform size. Mishandling often resulted in the appearance of mycelial-like bodies. The nutritional requirements of two L-forms were found to differ from those of their parent strains, from each other, and from those of a pleuropneumonia organism (134).

The sole chemical investigation of L-forms which was reported during 1953 has yielded unusual and very interesting results: Vendrely & Tulasne (430) compared the gross chemical composition of a normal and an 80 per cent L-type culture of *Proteus* on a per cent weight basis. They found that the L-culture contained only 0.20 per cent RNA compared to 8.31 per cent RNA in the control. DNA was down to 1.45 per cent from 3.28 per cent, while lipids were up at 24.3 per cent from 6 per cent in the controls.

Bacterial Genetics

Mechanisms for recombination of genetic material are apparently more numerous and diverse in bacteria than in any other group of organisms. This complex field is reviewed clearly and succinctly by Lederberg & Tatum (251). The results of the past year will be discussed below under separate headings which are used primarily for convenience. Their use should not be taken to imply the existence of definitive evidence establishing their biological distinctness.

Heterothallism in E. coli.—The simultaneous discovery in 1952 by Hayes in London, Cavalli in Milan, and the Lederbergs in Madison of heterothallism in the bacteria has had an understandable influence on research in bacterial recombination during the past year. Both Hayes (188, 189) and Cavalli, Lederberg & Lederberg (89) agree on the principal characteristics of the system. Two phenotypes exist which are distinguishable on the basis of the

following properties: F− strains are completely self-sterile; F+ strains are self-compatible, but show a considerably higher recombination frequency when crossed with F− strains.

The transmission and the expression of the F character display some unique characteristics. The F+ state is infectively transmitted to F− cells if the two types are grown together in broth, but it is not transferred in minimal medium, saline, or at low temperatures. Under conditions of rapid multiplication the transmission is extremely efficient, leading to the conversion of 50 per cent or more of the F− cells to F+ within 2 hr. So far as is known, no other genetic character is transferred at the same time. Once acquired, the F+ character is heritably stable. F− phenocopies can, however, be obtained by growing F+ cultures to high densities with vigorous aeration. The nature of the F+ agent and the mechanism of its transmission are at present uncertain, but it is definitely established that the F factor is independent of λ (89). Further, all attempts have thus far failed to transmit F+ by means of cell-free preparations of the most varied kinds. It would appear at present that cell to cell contact is necessary.

Cavalli has (88a) discovered a strain (Hfr) which yields 100 to 1000 times more recombinants in crosses with F− than does the normal F+ (Nfr) strain. A similar variant was subsequently isolated by Hayes (189), which appears to differ from the one found by Cavalli in that the expression of Hfr is confined to one linkage group (T, L, Lac).

The transmission of F+ and Hfr in crosses is quite complex. Depending on the particular F+ or Hfr strain under consideration, the progeny may be entirely converted to F+ (89, 189), partly converted to F+ (189), impervious to Hfr conversion (89), or almost completely changed to the Hfr condition (189). Linkage of Hfr and F+ to other genes appears to play a role (189), but the process as a whole is poorly understood.

The most important feature of the discoveries related to the F factor is the demonstration that the polarity of the parent strains has a profound effect on the genotype of the progeny resulting from a cross (89, 188, 189). The evidence is that the unselected genotype of the F− parent predominates in the progeny. As a consequence, unselected markers of the F− parent show spurious linkage to each other and to the F− selective markers. Knowledge that this is the case clearly necessitates a re-evaluation of existent interpretations of recombinant data which seek to establish linkage relations. This situation has led Watson & Hayes (446) to propose a hypothesis to explain the available information on recombination in K-12. Their model assumes the existence of at least three linkage groups, and postulates that zygotes are usually formed by the union of only one of the F+ "chromosomes" with the full gene complement of the F− strain. Many of the data are consistent with this hypothesis, although certain difficulties arise, particularly in connection with the Lac locus. More extensive data are required before a final resolution of this problem will be at hand.

Recent work with the Hfr strain has added a further complexity by the

finding that linkage values in crosses of Hfr to F− may be quite different from linkage in F+ × F− crosses (189). It has been observed (95) that the presence of 5 per cent KNO_3 or $MgSO_4$ has a stimulating effect (two- to threefold) on prototroph recovery in mating mixtures of K-12.

Transduction.—Zinder (480) provides an excellent summary of our knowledge of the phenomenon of transduction. Transduction of factors controlling flagellar antigens has been obtained using antiserum in semisolid agar to immobilize the recipient strain (249). Known serotypes, and new antigenic combinations which have no place in the Kauffman-White scheme, were thus constructed. Transductions of antigenic characters were not accompanied by detectable alterations of biochemical properties.

Stocker, Zinder, & Lederberg (411) report the results of their ingenious investigations of the transduction of flagellar characters in *Salmonella.* Using lysates from a number of O strains of *Salmonella typhimurium* as transducing principles for other O strains, they were able to demonstrate the existence of six separate factors capable of restoring motility to different nonmotile strains. The existence of at least six loci controlling motility could thus be inferred. The mutation of any one of these may cause the absence of flagella, thus masking H antigenic specificity. Transduction may therefore be used to reveal the type of previously untypable O isolates. The validity of typing results obtained by the transduction method was confirmed by independent evidence. Stocker *et al.* were also able to demonstrate the existence of two genes controlling flagellar motion and of a further gene concerned with the rapidity of swarming. In certain rare instances the simultaneous transduction of two flagellar characters were observed. In most cases of transduction, the lysogenic phage is also transferred along with the transduced character, but this is not always the case. Instabilities following transductions resulting in motile cells which give rise to nonmotile progeny has been discussed under the heading of gene action. A study of transduction, using Vi phage lysates in *S. typhosa,* has been reported (37, 38).

Transformation.—Pneumococcal transformation has been the object of a quantitative study by Stocker, Krauss, & MacLeod (410). Anti-R serum was used as a selective agent for transformed cells. The smooth cells formed were counted by the "probable number" method. The number of transformations during 30 min. of exposure to DNA was directly proportional to the amount of DNA added over a thousand fold range, suggesting that transformation results from the action of a single particle. It was calculated that 1.7×10^{-6} μg. of DNA can transform a single cell.

Four morphological cell types in pneumococcus involving combinations of the characters filamentous and encapsulated have been described (27) and their interconversion via transformation reactions demonstrated (28). Incubation with heterologous transforming principle resulted in the isolation of all the expected variants, excepting two of the four possible types involving double transformations.

Transformations of capsulated pneumococci have been reported (25). By

using doubly-marked killed and live strains of pneumococcus, and recovering recombinant transformed cells, Austrian (26) was able to demonstrate the reality of *in vivo* type transformation in pneumococci. Austrian & Colowick (29) have succeeded in converting slow-fermenters of salicin into fast fermenting types by exposure of the former to DNA extracted from the latter. Analogous results, although carried out under much more complex conditions were obtained with strains of *Staphylococcus albus* (119).

DNA isolated from crown-gall bacteria is capable of transmitting specific host virulence to nonvirulent strains of *Agrobacterium tumefaciens* and other members of this group. Once acquired, the virulence property is genetically fixed. The transforming principle is desoxyribonuclease sensitive (233). The meningococci have been added to the growing list of organisms susceptible to infectious heritable modifications by transforming principles (6).

Substantial progress in the knowledge of the transforming systems of *Hemophilus influenzae* is reported by a group which includes Zamenhof, Alexander, Leidy, Chargaff and collaborators (5, 253, 475, 476, 477). Transformations carried out with DNA from a cell type Smooth "a" (Sa) on cell type Sb produced a new cell, Sab, not found in nature. Transforming principle from Sab transforms rough cells to Sab, Sa, and Sb types. Further, the agent derived from transformed cell types is more active than the transforming principle from natural strains of equivalent phenotype (253). In another series of experiments (5) it was found possible to transform streptomycin-sensitive cells into streptomycin-resistant ones.

Important contributions to our knowledge of the chemistry of the active agent involved in transformations of *Hemophilus influenzae* were also made by this group (474, 475, 476). As in the case of other transforming principles, the agent is desoxyribonuclease-sensitive. Preparations which assayed at 96 per cent DNA were active transforming reagents at concentrations of 0.0004 μg./ml. (476). The minimal amount of DNA required to transform one cell in a thousand is five times the DNA content of the culture.

The type specific substance of *Hemophilus influenzae* type b has been purified and analyzed (477) and was found to be a polyribophosphate chain.

Tetrad Analysis and Haploid Genetics

Microbial genetics offers a uniquely useful tool for the analysis of meiosis in the form of data obtained with ascomycetes, basidiomycetes, and algae. In these forms the four products of a single meiosis can be isolated and characterized. Tetrad data possess obvious advantages in precision and statistical efficiency over those provided by the usual genetic material. Further, the results of tetrad analysis can be compared with recombinant data obtained in the same organism.

Both Papazian (325) and Spiegelman (393) have pointed out the incorrectness of the usually assumed factor for conversion of tetrad information into recombinant map distances and have derived the explicit relation which holds between the two mapping functions. The relations so derived in both

cases assume, however, the complete absence of chiasmata or chromatid interference and of sister strand crossing over. Barratt *et al.* (39) have attempted to take the existence of interference into consideration by inserting a correction factor empirically determined from existent data. Using the mapping function thus derived and the pooled information from several laboratories, they have constructed maps for the seven linkage group of *Neurospora crassa*. Perkins (329) has contributed an analysis of the statistical efficiency of linkage detection from tetrad and single strand data. He concludes that ordinarily linkage can be detected more efficiently from random strands.

A method for estimating the number of loci determining quantitative variation in haploid organisms has been proposed (94). Estimates are based on the variances with respect to the relevant quantitative character which are observed in F − 1 and backcross populations.

Fungi

Olive (323) has compiled a useful and exhaustive account of nuclear cytology in the fungi. Summaries of mutations, adaptive phenomena, and sexual factors in smuts (207), and of ecological adaptations in the fungi have (76) also appeared recently.

Phycomycetes.—Meiosis has been observed in various representatives of the *Saprolegniaceae* (479). A simple chemical test for sex in *Phycomyces blakesleeanus* is described (427). It consists in the addition of 30 per cent NaOH to thiamine-supplemented medium in which the organism in question has grown. A deep red color results with minus strains, but not with plus strains.

Accumulation of agents capable of stimulating oxygen consumption during sexual reproduction in the *Mucoraceae* has been observed (80, 81). This phenomenon is common to both heterothallic and homothallic species. Recent studies have implicated components of the Krebs cycle in the production of resistant sporangia in *Blastocladiella emersonii* (85). The ratio of orange to colorless plants could be affected by the use of the drug cycloheximide, but in contrast to observations in other *Blastocladiella* species, no sexual functions could be assigned to the different-colored plants (86).

Yeast.—Three papers have appeared during 1953 which should help to resolve the differences which have arisen in yeast genetics as a result of discordant results obtained in various laboratories. Taken together they provide definitive evidence for the mutability of the mating type locus in *Saccharomyces*. Ahmad (4) was able to isolate diploid cells from single spore cultures which no longer gave the mating type reaction, and which, upon sporulation, yielded spores of both mating types. Pomper & McKee (337) obtained the same result by a different method. They mixed complementary auxotrophic mutants of the same mating type. Dissection of the asci formed by the resultant diploid prototrophs revealed the existence of both mating types. Roman & Sands (363) isolated two types of diploids from single spore

strains. One was heterozygous for the mating type locus and therefore must have arisen as a result of the union between a mating type mutant and the parental strain. The other type was found to be homozygous for mating type and incapable of sporulation. Such diploids could mate either with haploids of the opposite mating type, or with diploids homozygous for the opposite mating type. Triploids and tetraploids may be constructed in this way. These findings offer simple and logical explanations for some of the aberrant asci which have frequently been reported in yeast genetics since its inception.

The introduction of a procedure permitting the genetic analysis of intact incomplete asci of yeast (63) and the discovery of strains yielding asci with linearly arrayed spores (187) may provide technically useful adjuncts to yeast genetics. The finding of more than four types of spores per ascus has eliminated extra mitoses as the sole explanation for the occasional occurrence of asci in *Saccharomyces cerevisiae* containing more than the usual four spores (270).

Winge & Roberts (456) have demonstrated that the strain "Yeast Foam" of *S. cerevisiae* carries R_2 of the polymeric series of genes controlling raffinose fermentation. They also provide evidence for close linkage between the raffinose gene R_3 and M_3, one of the four polymeric genes controlling maltose fermentation. Jinks (226) found that M_1 and R_1 are linked.

Disagreements concerning the dominance of the character "flocculence" in yeast may be explained by observations indicating that the expression of this character is strongly influenced by environmental factors (336). A study of the killing of budding yeast cells by ultraviolet irradiation led to the conclusion that yeast chromosomes are duplicated by the time buds can be detected (269).

Volume measurements of interphase nuclei in a series of polypoid stocks revealed considerable differences between strains of different ploidy (306). In the same study, a comparison of nuclear sizes of aerobically and anaerobically grown yeast failed to disclose differences. On this basis the author disputes previous claims that anaerobic growth results in endopolyploidy. Cytological observations on a diploid brewery yeast, which is believed to have a diploid chromosome number of two, suggest the possibility to the author that triploids may result from ordinary vegetative division (129). Diploid yeast, heterozygous for galactose fermentation, shows positive and negative sectoring when plated on galactose-eosin-methylene blue test plates subsequent to ultraviolet irradiation (219). The data suggest that homozygosity had developed with respect to both the gal locus and the mating type locus.

Lindegren (267) has published additional results to lend support to his "gene-conversion" hypothesis. In the present paper he seeks to avoid previous criticisms which invoke the possibilities of polyploidy, chromosomal elimination, and disomy. Two closely linked loci, each controlling a sugar fermentation, are used. In view of the radical nature of the hypothesis we feel, however, that conventional explanations for the aberrant segregations ob-

tained have not yet been ruled out with sufficient finality. Thus, the appearance of excess positives could be attributed to the occurrence of mutation and selection in the fermentation tubes which are used for the scoring of the phenotypes. Occasional recovery of excess stable negatives may perhaps be explained in terms of a suppressor phenomenon.

Ascomycetes.—Zickler's (478) pioneering investigations with *Bombardia lunata* have finally been published in detail. The paper was actually submitted for publication in 1940, but its appearance was delayed by the war, during which the author died. In addition to large numbers of original observations, the paper contains an extensive discussion comparing various ascomycetes with respect to their evolutionary relationships. The over-all pattern of the sexual behavior of *Bombardia* is very similar to that of *Neurospora*. However, the ascospores of the former are uninucleate, and the spermatia only function in fertilization. It appears further that the spore order in the *Bombardia* ascus is not as rigidly maintained as it is in *Neurospora*. Zickler was able to extract a hormone-like substance from the mycelium which functions in the attraction between the trichogynes and spermatia.

Singleton (387) presents a careful and thorough extension of McClintock's original observations on the cytology of *Neurospora crassa*. The existence of a seventh linkage group in the same organism has been confirmed (357). Thus, the correspondence between linkage group number and chromosome number is complete. Pittenger (331) has provided evidence which explains the genetic behavior of "pseudo wild types" in *Neurospora* in terms of disomics. "Pseudo wild types" are nutritionally prototrophic, but, in crosses to wild type, 50 per cent mutant and 50 per cent wild type progeny are recovered. The occurrence of the disomic condition is apparently widespread and was demonstrated in five of the seven linkage groups.

A phenomenon termed "semi-incompatibility" (fructifications in only one of the parental thalli) has been described in *Podospora anserina* (361). Genetic analysis of this phenomenon leads to the conclusion that it is affected by genes which are different from any of those controlling the "barrage" phenomenon discovered by the same author. There does seem to be a connection between the two phenomena, however, since semi-incompatibility does not occur in the absence of "barrage." A gene controlling "barrage" has been located on a chromosome which also carries a locus controlling color. Data concerning recombination between these loci and results obtained in earlier studies point to the existence of a strong and unique type of crossover interference, which, moreover, is markedly affected by temperature (302).

Extra-Sexual Recombination in Fungi.—The biological success of heterokaryosis as an alternative to the sexual process in providing genetic plasticity is evidenced by its widespread occurrence and its ability to replace sexuality in the *Fungi imperfecti*. In 1946, Pontecorvo (337a) pointed out the great potentialities inherent in heterokaryons as material for fundamental biological investigations. The results of the detailed and ingenius investigations of Pontecorvo and his colleagues over the past five

years have now made their appearance in a series of papers which deserve to be ranked among the significant achievements of microbial genetics. As a result of this work, the *Fungi imperfecti* have now become accessible to genetic investigations, and a new dimension has been added to genetic experimentation in the perfect fungi. Finally, these studies have provided an experimental system uniquely suitable for the investigation of somatic recombination.

Perhaps the most important aspects of these researches stemmed from Roper's (364) demonstration that heterozygous diploids can be produced in the filamentous fungi. Normally the conidia of *Aspergillus nidulans* are haploid and uninucleate. Employing complementary auxotrophic-deficient stocks and plating on minimal medium, prototrophic colonies could be recovered at a low frequency. That these prototrophic strains indeed represented diploids rather than heterocaryons could be confirmed in various ways. For one, heterocaryons always segregate the component types in their conidia. Thus if a mycelium contains a mixture of nuclei bearing color markers (e.g., white and yellow), the conidia produced by the heterocaryon would be either white or yellow, since they are haploid. If, on the other hand, the prototrophy were caused by diploidisation, conidia produced would be diploid and therefore of the wild type color (green). The latter condition was observed. Further evidence for diploidy was obtained from the behavior of the prototrophic strains in crosses. They frequently yielded 16 rather than the usual 8 spored asci and the resultant ascospores showed an abnormally low viability. Finally, cytological observations also provided clear evidence for diploidy.

Prompted by the classic investigations of Stern (404), a search was made for the existence of somatic crossing over in diploids constructed by Roper's technique. Since mitotic chiasmata occur with an estimated frequency of 1 in 200, this search was soon crowned with success. Having established the characteristics of diploid organisms and verified the occurrence of somatic recombination, attempts to duplicate these results in imperfect fungi were initiated. These were immediately successful in both *Aspergillus niger* (340) and *Penicillium chrysogenum* (341).

In the course of investigating the behavior of the diploids produced in *A. niger* (340), conidia were noted which were smaller than normal and which did not segregate further as did the normal sized diploid conidia. In a subsequent investigation with *A. nidulans* Pontecorvo *et al.* (339) returned to this problem and demonstrated that these abnormally small conidia, arising from diploid mycelia, were haploid. Somatic recombination in *A. nidulans* could thus be attributed to the simultaneous occurrence of two processes: recombination strictly analogous to the phenomena observed by Stern (404) and irregular distribution of whole, unrecombined chromosomes at mitosis, leading to haploid nuclei recombining markers on different chromosomes. The latter process provides a unique and very useful tool for the determination of linkage groups. Somatic recombination opens up the possibility of

studying the genetics of asexual forms, and, in addition, permits the performance of experiments unavailable to Stern (404), in that the cells which arise from the somatic crossing over process can be subjected to further genetic analysis.

An interesting phenomenon termed "relative heterothallism" was uncovered in perithecial analysis in *A. nidulans* (338): In many crosses carried out with balanced (about 1:1 nuclear ratio) heterocaryons, the incidence of perithecia containing markers from both component strains was almost 100 per cent while it should have been about 50 per cent on a chance basis (only two nuclei give rise to a perithecium in this organism). While the mechanism of relative heterothallism is unknown, it greatly facilitates genetic analysis.

The life cycle of *Aspergillus nidulans*, the methodologies, and many of the data described in the foregoing are detailed in an extensive paper (338).

HETEROCARYOSIS.—Genetic factors controlling ability to form heterocaryons have been found in *Neurospora*. Stable heterocaryons apparently can be made only if each member carries the proper alleles of two independently segregating genes designated as het-1 and het-2. With this condition satisfied, even strains of opposite mating type will form heterocaryons (159). A method for determining the proportions of nuclear types in heterocaryons is proposed (345) which depends on the assumption of random distribution of nuclei. The data are consistent with the theory in only one case, which is not surprising in view of the previous evidence of nonrandomness in the distributions of nuclei in mycelia. In *Aspergillus nidulans*, where striking color markers and uninucleate conidia greatly facilitate heterocaryon analysis, "heterocaryons" are known to consist in part of homocaryotic hyphae in syntrophy and in part of heterocaryotic hyphae. The relationship between these components is not constant. Moreover, the nuclear ratio determined from a conidial population is different from that observed with an ascospore sampling from the same thallus (338).

Fixed nuclear ratios in heterocaryons of *Neurospora* composed of argininеless and lysineless strains have been demonstrated. This can be explained in terms of the observations that lysine inhibits the argininеless mutant and arginine inhibits the lysineless mutant. That this cross inhibition constitutes the controlling mechanism is supported by the behavior of heterocaryons involving the same biochemical deficiencies, but with mutant strains resistant to the heterologous amino acids. In such heterocaryons a wide range of nuclear ratios is observed (395). Early work on the properties of heterocaryons in the aspergilli has been confirmed by a recent publication (354).

Basidiomycetes.—The tetrapolar sexuality which characterizes a large number of basidiomycetes (422) has long been the object of intense investigation. In this system, mating occurs only between nuclei heterozygous for two independent mating type loci. Of particular interest has been the "Buller phenomenon," in which an apparent breakdown of this requirement occurs in noncompatible matings between a dikaryon and a monokaryon. Recent results obtained with this experimental system, including unpublished work

by Papazian and others, are summarized by Raper (353). Suggestive evidence for the interpretation of the Buller phenomenon in terms of somatic recombination is given. Further support for this view is provided by Terra's (423) investigations with several basidiomycetes. Other investigations of the dikaryotic phase in the basidiomycetes (21, 154) have already been discussed in connection with the problem of cytoplasmic inheritance.

Algae

There has been a renewal of activity in the investigation of *Chlamydomonas*. The isolation of mutants including biochemical auxotrophs has been described (261, 321). A detailed description of the methods employed and of mechanical procedures useful for the isolation of zygotes are given (261). On the basis of the analysis of seven genetic types, Lewin (261) concludes that regular Mendelian segregation is occurring and that crossing over takes place in the four strand stage. Sager & Granick (374) report that nitrogen depletion is an important factor in gamete formation. Stich & Hammerling (408) have studied the incorporation of P^{32} in young and older nuclei and nucleoli of *Acetabularia*. Using micromanipulation techniques, they succeeded in isolating nucleoli which were apparently free of contaminating materials. They could establish that 80 per cent of the incorporated P^{32} of the nucleus was localized in the nucleolus, and that the nucleoli of young algae assimilated as much P^{32} as those of older plants although they were only about one sixth as large. Papers relating to the role of the nucleus in *Acetabularia* development have already been discussed (60, 61, 70, 407). Additions to our knowledge of the life cycles of two other algae (186, 283) may eventually permit their use as materials for genetic analysis.

Protozoa

The antigenic structures of three serotypes of *Paramecium aurelia* were compared by studying reactions between fresh and ground, lyophilized animals of each serotype on the one hand, and antisera against fresh animals of each serotype on the other (429). It was found that reactions between fresh cells and their antisera were highly specific, but that extensive cross reactions occurred between lyophilized cells and the sera. Additional separations of antigens could be achieved by fractionation of the homogenates.

A multiple mating system involving seven mating types has been found in *Tetrahymena pyriformis* (312). Any mating type will mate with any other, but not with itself. The progeny from a given cross may be of several or perhaps all the mating types. As in mating type determination in *Paramecium* (310), genic, cytoplasmic, and environmental influences appear to play a role (311). (See also discussion of cytoplasmic inheritance.)

Observation of the fate of the macronucleus of the ciliate *Epistylis articulata* suggests that it breaks up into smaller fragments which eventually become Feulgen negative (381). In the process of losing Feulgen positivity the fragments stain in a complementary way with pyronin-methyl green which

is presumably specific for RNA. Digestion with ribonuclease results in a loss of the staining properties. On the basis of microscopic evidence Hartmann (184) confirms his earlier conclusion that the macronuclei of several *Radiolaria* species originate through an endomitotic process. Later the macronucleus breaks down into component nuclei which can still divide. On the basis of microscopic observations on other *Radiolaria* species, Grell (170, 171) concludes that the macronucleus in question does not consist of nuclei, but of multiple-strand chromosomes. A cytological description of conjugation and macronuclear division in *Ephelota gemmipara* is given by the same author (169).

Population Genetics and Evolution

Problems of evolution and microbial population changes in natural surroundings are discussed by Stanier (396). Levine (257a) points out that the mate-killer phenomenon in *P. aurelia* is a powerful mechanism for species isolation. Pontecorvo (338) discusses the functioning of heterocaryosis in the preservation, selection and recombination of spontaneous mutants.

The introduction of various chemotherapeutic and antibiotic agents has had a profound effect on the incidence of resistant bacteria. A number of studies (127, 143) have demonstrated a dramatic rise in the proportion of penicillin-resistant staphylococci in the years from 1949 to 1953. The same phenomenon was observed with respect to the occurrence of mutants resistant to other newly introduced antibiotics (127). A study of the distribution of penicillin-resistant mutants among hospital staffs (437) and infants delivered in hospitals (277) demonstrates clearly that hospital personnel represent a dangerous reservoir of penicillin-resistant organisms.[3]

The use of salt agglutination, which distinguishes between virulent and avirulent types of *P. tularensis*, has permitted a study of the population changes during laboratory transfer (30, 468). The observation that highly resistant strains of tubercle bacilli can be obtained from human beings treated with streptomycin was found not to apply to either guinea pigs, mice, or rabbits (458). Reversible changes from smooth to rough morphology in coliform bacteria were found to be very strongly influenced by the growth medium. While the techniques employed interdict any conclusions, the mechanism of these variations is probably selectional (240).

NATURE AND PROPERTIES OF THE GENETIC MATERIAL

Fine Structure, Molecular Structure, and Chemical Composition of the Hereditary Material

It is rather generally accepted that genetic continuity resides either solely or in part in DNA. Evidence for this view comes from the straightforward relationship between ploidy and DNA content of cells, the identification of highly purified DNA as the active agent in transformations, and the role of

[3] See also the chapter on "Changes in the Host Flora Induced by Chemotherapeutic Agents" by McCoy in this volume.

DNA in phage genetics (193). That DNA may not be the only genetically competent material is indicated by its absence from some plant viruses.

DNA structure.—The structure of DNA which has been proposed by Watson & Crick (443, 444, 445) possesses profound genetic implications which have already been widely discussed among microbial geneticists (441a). Briefly, DNA is pictured as consisting of two chains of polynucleotides coiled around a common axis and joined by hydrogen bonding between the nucleotide bases. The phosphate-sugar backbones run in opposite directions along the two helices, with the purine and pyrimidine bases facing toward the interior of the coil, while the phosphate groups are on the outside. The spatial arrangement is such that a given base in one chain determines the nature of its complementary base in the other. The structure was based on available crystallographic, physico-chemical and analytical information. Supporting x-ray data were published simultaneously (145, 146, 455). Additional x-ray data (20), electron micrograph pictures (149, 226a), and especially a large body of recent chemical-analytical data provide added evidence for the proposed structure. The previously mentioned compulsorily complementary feature of the model requires a 1:1 ratio between complementary bases, specifically between adenine and thymine and between guanine and cytosine (or 5-methyl-cytosine, or 5-hydroxymethyl-cytosine). These relationships have already been established in the course of the past two years by the brilliant analytical work of the Chargaff group and of others and have been amply corroborated this year in microbial material (82, 99, 156, 464), as well as in individual fractions of DNA from higher organisms (75, 93). While the adenine-thymine and guanine-cytosine (or cytosine-derivative) ratios in DNA from different biological sources are always close to unity, considerable variation can and does exist in the ratios of bases not complementary according to the Watson-Crick model (464). In closely related organisms, however, the latter ratios are similar (99, 156, 464). The finding of a new species-specific base, 5-hydroxymethyl-cytosine, in T2, T4, and T6 has already been mentioned (465). This base has not been found elsewhere in nature or in any other virus.

Plant virus RNA analyses.—Analytical work has been done on plant virus RNA and here also the base-ratios in different virus species were different, while independent preparations of a given species were the same (124). Careful work is beginning to reveal suggestive regularities in RNA structure (289). The purine antagonist, 8-azaguanine, has been found incorporated in the RNA of tobacco mosaic virus from plants sprayed with this agent (289).

Virus protein.—In contrast to the apparent similarity in the nucleic acid composition of related viruses, the amino acid composition of eight mutant strains of tobacco mosaic virus was not the same. Five different patterns could be distinguished (66). Further progress in the determination of the amino acid sequence of tobacco mosaic virus protein is reported by Schramm & Brannitzer (380) who document their belief that the structure of this entire protein may soon be unravelled. The amino acid composition of

T3 protein has been found to be the same under various growth conditions (148).

Genetic evidence relevant to the structure of DNA.—Information relating to the structure of genetic material has also been obtained in radioactive tracer experiments. Friedrich-Freksa & Kaudewitz (153) exposed *Amoeba proteus* to P^{32} and then followed the appearance of lethals in 100 subsequent generations and 30,000 individuals. The per cent lethals reached a maximum in the fifth generation and again in the thirty-first generation. The first maximum corresponds to that observed in controls subjected to P^{32} emissions from without. The second can be explained if one assumes a sixteen strand chromosome stage in the original irradiated amoebae; damage to individual strands due to the $P^{32} \rightarrow S^{32}$ transformation would then be expressed most strongly in the thirty-fifth generation.

The differential sensitivity of different phage loci to the effect of the $P^{32} \rightarrow S^{32}$ transformation has already been cited (402). In whole phage, only one in about thirteen such transformations is lethal (401). Purely genetic information concerning the fine structure of genes has been obtained by Pittenger (331), who analyzed numerous examples of pseudoallelism in *Neurospora*.

An interesting demonstration of the persistence of P^{32} label and thus, perhaps, of genetic material has been achieved by Kaudewitz (229). *Paramecium aurelia* were allowed to ingest bacteria highly radioactive with P^{32}, and to empty their vacuoles of radioactive waste products. The distribution of tracer from individual paramecia to their daughter cells and to further generations was then measured. It was found that the sixteen animals of the the fifth generation contained 96 per cent of the activity of the ancestral cell from which they were derived.

Incorporation of P^{32} into the DNA of *E. coli* was found to be independent of the phosphate concentration in the medium. Under the same conditions, the RNA content of the cells varied rather widely (369).

Spontaneous and Induced Change of Genetic Material

Killing and mutagenic effects of radiation.—Relatively few of the papers published in the field of radiobiology in 1953 are concerned primarily with the statistical killing effect of the various mutagenic agents.

Wood (460) and Lucke & Sarachek (274) obtained reproducible survival curves with x-ray irradiated haploid and diploid yeast which were interpretable on the basis of the known ploidy of the cells. Ultraviolet irradiation of the same material, however, yielded complex results (376). Extensive experiments with haploid and diploid yeast, utilizing a wide range of ionizing radiation, led Zirkle & Tobias (481) to the conclusion that a simple target theory cannot explain all the observed results. The data are interpretable if the production of a diffusible substance is postulated. Nybom (321) observed in experiments with *Chlamydomonas* and yeast that the incidence of zero-point killing in ultraviolet irradiation experiments was relatively much higher

than in x-ray irradiation experiments. This was interpreted as an indication that ultraviolet killing is in large part nongenetic, while x-rays affect primarily the genome.

X-rays of different energies produced identical killing curves in *E. coli* (273). Linear dose-effect curves were obtained in ultraviolet irradiation of *Tetrahymena* (290a). Good agreement was obtained in some cases between the target volume calculated from radiation data and known nucleic acid volumes of ten different viruses (138). Ultraviolet action spectra for the killing of dry phage (144) and for killing and the induction of biochemical mutations in bacterial spores have been delineated (302a). The mutation and killing spectra differ somewhat from each other and from the ultraviolet absorption spectrum of the spores.

Bachofer *et al.* (31) performed experiments designed to identify and separate direct and indirect radiation effects. Dry T1 phage was x-rayed at temperatures ranging from 77°K. to 310°K. and a temperature effect amounting to an activation heat of 1100 calories was calculated. Assuming all indirect effects to be negligible at 0°K., the residual radiation damage attributable to direct effect could be estimated by extrapolation of the temperature coefficient curve to 0°K. The relationship between the number of γ-radiation-resistant bacteria recovered, and the dose of mutagenic radiation delivered, has been investigated (155).

Environmental effects on mutation and survival.—(*a*) *Oxidation state:* The expression of the effect of oxygen in increasing radiation damage depends largely on the absence of antiperoxide agents. Failure to observe mutagenic effects may thus be traced to high catalase content of bacteria (176). In a direct comparison, differences in ultraviolet sensitivity could be attributed to differences in catalase content (298). Peroxidase also counteracts ultraviolet damage (248). Bacteria in the medium forestall nongenetic damage to *Paramecium* by ultraviolet, presumably through their antiperoxide action (232). Kaplan (227) failed to observe oxygen effects in experiments with *Serratia marcescens*, but the role of catalase or peroxidase was not investigated in this system. Oxygen increased x-ray destruction of kappa in *Paramecium* (160) and drastically reduced the survival of x-irradiated *E. coli* B/r. Various types of chemicals counteracted this latter effect (206). The same substances also proved effective in protecting *Chlamydomonas* from x-ray and ultraviolet damage, but an oxygen effect was only noted in x-ray experiments (321). Aerobic conditions may stimulate Fe^{++}-induced reversion from streptomycin dependence to independence in *E. coli* (88).

(*b*) *Media and chemicals:* Some of the effects of different media on survival after irradiation can also be attributed to peroxide effects. Thus, incubation in iodoacetate prior to ultraviolet treatment increases survival of *Streptomyces* spores, an effect which is antagonized by peptone (440). Growth or incubation of bacteria in azide also increases survival after ultraviolet irradiation, but photoreactivability is reduced simultaneously (51). A clear interrelationship between peroxide production of different media, killing,

and heat reactivability emerges from the extensive work of Harm & Stein (180, 181, 399). The greater the boost in survival of irradiated or untreated *E. coli* at 44.5°C. against 37°C. on a given medium, the more drastic the kill by these same media when used in indirect inactivation experiments (ultraviolet-irradiation of media) (399). Differences among complete media amounting to a survival factor of 25 were observed, and even the length of autoclaving affected survival (180). A very different mechanism for a medium effect was indicated in experiments by Bachofer & Pottinger (32). Here, protection of phage T1 against x-ray damage by high concentrations of ammonium sulfate was attributed to dehydration of the virus protein. Failure to form visible colonies on adaptive substrates by cells viable on constitutive ones can be explained by the inactivation of the mechanisms of adaptive enzyme formation (318). Unexplained effects of glucose starvation and α-CH_3-glucoside supplementation are reported (133, 441).

(*c*) *Temperature effects:* Some seemingly contradictory temperature effects may become reconciled by the demonstration that mutants of a given organism show drastically different, even opposite, mutation and survival effects at different temperatures (8, 180). Further, the pronounced temperature effects are generally observed only on organic media (180, 206).

The ultraviolet sensitivity of B and B/r are indistinguishable if these strains are plated at 44.5°C. instead of 37°C. after irradiation. The capacity to be heat reactivated disappears within three hours of irradiation (180, 398). Heat, and also cold reactivation occurs at temperatures outside the physiological range (181, 397), but at the extreme temperatures these effects are generally masked by killing (181). The extent of reactivation has two maxima near 10°C. and 44.5°C. and drops to a minimum near 25°C. in the case of *E. coli* B (180). Light reactivation produces effects of a magnitude comparable to heat reactivation in *E. coli* strains B and B/r, but they are not the same (181). A theoretical model incorporating the main inactivation and reactivation results is presented by Stein & Harm (400).

The recovery of mutants is also greatly affected by the post-ultraviolet irradiation temperature. At 16°C. only 5 per cent as many T1-resistant mutants are recovered as at 37°C. This temperature effect is confined to the first post-irradiation division (457). Evidence that a decrease in mutant yield at 15°C. is coupled with a higher killing rate is consistent with several of the above mentioned findings (54). Both the killing and mutagenic effects of Fe^{++} on *E. coli* are greater at 1°C. than at 37°C. (87).

An effect of pre-irradiation temperature treatment is described by Langendorff, Langendorff & Sommermeyer (246). These authors noted that cultures of *E. coli* B are more sensitive to x-rays if held at 44.5°C. before irradiation that if held at 37°C.

(*d*) *Light effects:* An increase in the growth lag produced by ultraviolet may be reversed by visible light (45). A powerful photoreactivation effect has been observed in *Chlamydomonas*. At the same time, light sensitizes this organism towards x-rays (321).

(*e*) *Ultraviolet reactivation:* When phage is inactivated with ultraviolet, x-rays, or N-mustard, a substantial increase in plaque-forming capacity is obtained if the sensitive bacteria are treated with ultraviolet prior to or during the infection. This effect is distinct from photoreactivation which can also be demonstrated (451).

(*f*) *Plating density:* Dense seeding of plates in mutation experiments may result in the suppression of mutant organisms and in erroneous conclusions regarding mutagenic effects (378, 405). Claims that this phenomenon has been neglected in older work (225) are refuted (371).

Physiological variation and mutation and survival.—If *E. coli* B is grown in broth for four days instead of one, a fourfold increase in resistance to ultraviolet is obtained (398). Similarly, young yeast cultures are more sensitive to x-rays than older ones (460). These effects are reminiscent of the increase in resistance to streptomycin with age which has been noted (69). A certain colorless spontaneous variant occurs with a frequency of about 30 per cent in young cultures of *Serratia*, while it appears with less than 1 per cent frequency in older cultures (245). Striking cyclical change in sensitivity to ultraviolet- and α-radiation has been found by Houtermans (209) to be associated with stages in the division cycle in synchronously dividing microcultures of bacteria.

Nonlinear survival curves, especially "tailing" at high doses, can sometimes be attributed to inhomogeneous populations of cells. In experiments with *Glomerella* conidia, Markert (288) was able to distinguish the resistant portion of the population from the sensitives by inspection. Analogous inhomogeneities have been noted by several investigators during the past year (132, 375, 460).

Genetic parameters.—(*a*) *Mutability:* An extensive analysis of existing quantitative descriptions of mutation frequency in microbial populations and methods for estimating the effects of various assumptions on such phenomena as phenomic lag and distribution of the mutational event during the division cycle have been presented by Armitage (18, 19).

Several studies of mutability of individual loci have appeared in 1953. Giles, Kolmark and their associates have both studied mutability of various alleles at an inositol and an adenine locus in *Neurospora* (117, 161, 236, 237, 238). Fifteen inositolless alleles could be classified into five different groups on the basis of their spontaneous and radiation-induced reversion rates (117), while five adenineless alleles fell into two groups (161). Many chemical mutagens were screened for their efficiency in inducing reversions (238). Striking evidence for specific mutagenic effects has been obtained. In a double (adenineless-inositolless) mutant of *Neurospora*, the ratio of ultraviolet-induced reversion at the adenine locus to ultraviolet-induced reversion at the inositol locus was 0.65. The corresponding ratio for diepoxybutane-induced reversions was 494 (237). In a double mutant of *E. coli* a histidine locus was found refractory to the mutagenic action of $MnCl_2$, while appreciable effects were obtained at a locus for streptomycin dependence (116).

Pontecorvo (338) compares the incidence of different biochemical muta-

tions in mutant-isolation experiments in *Penicillium*, *Neurospora*, *Aspergillus* and *Ophiostoma*. He notes that in all these organisms, mutation to arginine, lysine, and adenine deficiency is common. On the other hand, only *Penicillium* and *Neurospora* show a high frequency of methionine-cysteine mutants, while *Aspergillus* and *Ophiostoma* mutate frequently to a parathiotrophic condition, or one incapable of using hexavalent sulfur.

The spontaneous occurrence of a specific lethal mutation in *Neurospora* with a frequency of over 0.5 per cent of random individual isolates was observed (24). Theophylline was found to stimulate mutation to T5 resistance in *E. coli* B, but not mutation to T6 resistance (252). A study of reversion frequency in 70 independent isolates of streptomycin-dependent mutants of *E. coli* revealed a wide spectrum of mutability (243).

(*b*) *Mutagenesis:* Raper (353) reports on a phenomenon labeled "heterocaryotic mutagenesis" in which a 100,000-fold increase in mutability with respect to certain characters is observed on establishing heterocaryons.

Newcombe (315a) has analyzed the behavior of unstable strains of *Streptomyces* which were obtained spontaneously and with a very high frequency after ultraviolet- or γ-irradiation. The inheritance patterns of the instabilities suggested that the phenomenon in question may correspond to chromosomal aberrations of higher organisms.

Alpha hydroxy acids, which show the L-tartaric acid configuration around the alpha carbon, apparently increase the reversion rate of a glycineless *E. coli* mutant to glycine independence. Their enantiomorphs are inactive. Further, some of the acids yielded partial reverted types, while others gave full reversion (461).

A remarkable mutagenic effect of chloromycetin and serum on *E. coli* originally discovered by Voureka, has been further investigated by Manten & Rowley (285). Peculiar types of mutants with a spectrum of modified properties were obtained, as well as large numbers of ordinary auxotrophs.

Negative results were obtained by Ryan *et al.* (372) in attempts to induce mutations with absorbed antisera. A hypothesis that the mutagenic effect of theophylline (1,3-dimethylxanthine) is attributable to its incorporation into DNA was not substantiated in tracer experiments with C^{14} (235). Experiments designed to select *Neurospora* mutants resistant to various poisons gave completely negative results (41). On the other hand, the presence of sulfanilamide in the crossing medium is reported to select for resistant organisms or even to induce sulfanilamide resistance in *Neurospora* (230).

Failure to obtain dominant lethals in *Paramecium* was attributed to selective mating by undamaged nuclei only (231). The production of recessive lethals in yeast by x-ray (303) and of N-mustard induced reversions and suppressors in *Neurospora* (406) was reported. Azide stimulated mutation to streptomycin resistance (51).

The stimulation of mutation rates in phages has in the past been difficult to achieve. Three different approaches which have been successful were reported in 1953. In ultraviolet reactivation experiments, an appreciable

proportion of the reactivated phage are mutant (451, 453). If T2 is grown in streptomycin-sensitive cells in the presence of low levels of streptomycin, an increased number of host-range mutants is obtained (140). The transient presence of from 3 to 4 γ per ml. of proflavine during early development of T2 results in the production of a phage population which contains up to 20 per cent plaque-type mutants (113, 114).

(*c*) *Phenotypic delay:* One of the questions most relevant to the study of phenotypic delay is whether mutation rate is a function of division frequency. By studying the appearance of mutants in populations dividing at very different rates, Labrum (244) and Lee (252) both confirm previous investigations which indicated that rate of mutation is constant with time. A considerable lag between the application of the mutagen and the appearance of the first T5-resistant mutants was observed under all growth conditions (244).

Demerec & Cahn (116) studied the delayed appearance of mutations at five loci of *E. coli*, using various mutagens. Each locus had a characteristic delay which was independent of the mutagen used. These results appear to rule out any simple "segregation delay" or "irregularity delay" (315) as explanations of phenotypic lag. That there may be a delay in the expression of the phenotype following change in genotype is shown clearly by the results of Chao (92) which have already been cited.

Newcombe (315) estimates an overall phenotypic delay (attributable to all causes) as ranging between a 2^3- and 2^7-fold population increase. He provides evidence that the inordinately long delay (2^{13}-fold increase) observed in the expression of resistance to T1 results from experimental artefacts.

Witkin (457) has found that phenotypic delay of mutation of *E. coli* to T1 resistance has a Q_{10} of about 3. The Q_{10} of growth and of spontaneous mutation are the same. Labrum (243) measured the phenotypic delay of reversion from streptomycin-dependence in *E. coli* while Kubitschek & Bendigkeit (241) studied the delay in the appearance of T5-resistant mutants of *E. coli* in a chemostat and found that 90 per cent of the phenotype was expressed within five generations.

METHODS

Several methods of interest to workers in various branches of microbial genetics have been developed during the past year.

In the bacterial viruses Bresch (72) has described a "technicolor" mutant scoring technique. In this procedure two dyes are incorporated into the agar medium, and mutants are differentiated from one another on the basis of the color and the turbidity of the plaques they produce. Benzer & Jacob (50) have employed cyanide (0.01 M), Stent (401) storage at −196°C. to achieve reversible interruption of virus development.

Dulbecco & Vogt (128) have developed a plaque technique which promises to confer on animal virology all of the technical advantages which have made possible the recent dramatic advances in the bacterial virus

field. The use of de-embryonated eggs which permit easier handling of viruses is introduced as a more convenient system for the growth of influenza virus (79).

Levine *et al.* (257c) describe a hemagglutinin method for titration of animal viruses which measures the number of virus particles fairly directly and also rapidly.

A plating method is proposed for screening ultraviolet-induced mutants of *Streptomyces griseus* which produce antibiotics active against *Bacillus subtilis* (330). Pontecorvo and his co-workers (338) have developed a variation of the starvation method for mutant enrichment to a point where auxotroph yields of as high as 60 per cent can be obtained with *Aspergillus nidulans*. A partial separation of bacterial variants in a culture has been achieved by an electrophoretic method (183).

By maintaining an exclusively conidial growth habit in *Ophiostoma*, Hofsten *et al.* (205) have succeeded in growing this fungus in continuous culture in a specially adapted chemostat. A refinement of the chemostat which permits automatic recording of growth rates has been described (11).

A new and ingenious method for controlling growth rates in bacterial cultures has been devised by Jacob (214). In this method, a carbon or nitrogen source essential to growth is made available to the bacterial culture at a constant rate by enzyme action. In particular, Jacob achieved widely varying growth rates with cultures of lactose-negative *E. coli* by the addition of graded amounts of lactase to the lactose growth medium. Similarly, a controlled nitrogen release was affected by the system urease-urea.

Bevan proposes a method for the rapid accumulation of linkage information in yeast which involves the analysis of colonies resulting from the germination of undissected 1-, 2-, and 3-spored asci (63). The use of cement blocks is suggested as a substitute for the usual gypsum blocks for ascospore production (182).

LITERATURE CITED

1a. Abraham, E. P., *Adaptation in Microorganisms*, Chap. 9 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
1. Adams, M. H., *Ann. N. Y. Acad. Sci.*, **56**, 442 (1953)
2. Adams, M. H., *Ann. inst. Pasteur*, **84**, 164 (1953)
3. Adelberg, E. A., *Bact. Revs.*, **17**, 253 (1953)
3a. Adelberg, E. A., and Umbarger, H. E., *J. Biol. Chem.*, **205**, 475 (1953)
4. Ahmad, A., *La Cellule*, **55**, 235 (1953)
5. Alexander, H. E., and Leidy, G., *J. Exptl. Med.*, **97**, 17 (1953)
6. Alexander, H. E., and Redman, W., *J. Exptl. Med.*, **97**, 797 (1953)
7. Ames, B. N., Mitchell, H. K., and Mitchell, M. B., *J. Am. Chem. Soc.*, **75**, 1015 (1953)
8. Anderson, E. H., and Billen, D., *Records Genetics Soc. Am.*, **22**, 63 (1953)
9. Anderson, E. S., and Felix, A., *J. Gen. Microbiol.*, **9**, 65 (1953)
10. Anderson, E. S., and Felix, A., *J. Gen. Microbiol.*, **8**, 408 (1953)
11. Anderson, P. A., *J. Gen. Physiol.*, **36**, 733 (1953)
12. Anderson, T. F., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 197 (1953)

13. Anderson, T. F., Rappaport, C., and Muscatine, N. A., *Ann. inst. Pasteur*, **84,** 5 (1953)
14. Appleby, J. C., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 19 (1953)
15. Appleyard, R. K., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 95 (1953)
16. Arima, K., Komagata, K., and Nakamura, H., *J. Agr. Chem. Soc. Japan*, **27,** 389 (1953)
17. Arima, K., Nakamura, H., and Komagata, K., *J. Agr. Chem. Soc. Japan*, **27,** 345 (1953)
18. Armitage, P., *J. Roy. Statistical Soc.*, [B]**14,** 1 (1952)
19. Armitage, P., *J. Hyg.*, **51,** 162 (1953)
20. Arndt, U. W., and Riley, D. P., *Nature*, **172,** 803 (1953)
21. Aschan, K., *Svensk Botan. Tidskr.*, **46,** 367 (1952)
22. Aschan, K., and Norkrans, B., *Physiol. Plantarum*, **6,** 564 (1953)
22a. Atwood, K. C., *Genetics*, **37,** 564 (1952)
23. Atwood, K. C., and Mukai, F., *Proc. Natl. Acad. Sci. U. S.*, **39,** 1027 (1953)
24. Atwood, K. C., and Mukai, F., *Records Genetics Soc. Am.*, **22,** 64 (1953)
25. Austrian, R., *Bull. Johns Hopkins Hosp.*, **90,** 170 (1952)
26. Austrian, R., *Bull. Johns Hopkins Hosp.*, **91,** 189 (1952)
27. Austrian, R., *J. Exptl. Med.*, **98,** 21 (1953)
28. Austrian, R., *J. Exptl. Med.*, **98,** 25 (1953)
29. Austrian, R., and Colowick, M. S., *Bull. Johns Hopkins Hosp.*, **92,** 374 (1953)
30. Avi-Dor, Y., and Yaniv, H., *J. Bact.*, **66,** 1 (1953)
31. Bachofer, C. S., Ehret, C. F., and Powers, E. L., *Proc. Natl. Acad. Sci. U. S.*, **39** 744 (1953)
32. Bachofer, C. S., and Pottinger, M. A., *J. Gen. Physiol.*, **36,** 345 (1953)
33. Barber, M., *J. Gen. Microbiol.*, **8,** 111 (1953)
34. Barber, M., *Adaptation in Microorganisms*, Chap. 10 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
35. Barber, M., *J. Gen. Microbiol.*, **8,** 104 (1953)
36. Barksdale, W. L., and Pappenheimer, A. M., *Federation Proc.*, **12,** 435 (1953)
37. Baron, L. S., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 271 (1953)
38. Baron, L. S., Formal, S. B., and Spilman, W., *Proc. Soc. Exptl. Biol. Med.*, **83,** 292 (1953)
39. Barratt, R. V., Newmeyer, D., Perkins, D. D., and Garnjobst, L., *Advances in Genetics* (In press)
40. Barrett, J. T., Larson, A. D., and Kallio, R. E., *J. Bact.*, **65,** 187 (1953)
41. Bartmann, K., *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmakol.*, **218,** 327 (1953)
42. Bautz, E., and Marquardt, H., *Naturwissenschaften*, **40,** 531 (1953)
43. Bautz, E., and Marquardt, H., *Naturwissenschaften*, **40,** 531 (1953)
44. Beale, G. H., *Adaptation in Microorganisms*, Chap. 17 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
45. Beckhorn, E. J., *J. Bact.*, **63,** 687 (1953)
46. Beljanski, M., *Compt. rend.*, **236,** 1102 (1953)
47. Beljanski, M., *Ann. inst. Pasteur*, **84,** 757 (1953)
48. Beljanski, M., and Grumbach, F., *Compt. rend.*, **236,** 2111 (1953)
49. Benzer, S., *Biochim. et Biophys. Acta*, **11,** 383 (1953)
50. Benzer, S., and Jacob, F., *Ann. inst. Pasteur*, **84,** 186 (1953)
51. Berger, H., Haas, F. L., Wyss, O., and Stone, W. S., *J. Bact.*, **65,** 638 (1953)
53. Berger, H., and Wyss, O., *J. Bact.*, **65,** 103 (1953)

54. Berrie, A. M. M., *Proc. Natl. Acad. Sci. U. S.*, **39**, 1125 (1953)
55. Bertani, G., *Ann. inst. Pasteur*, **84**, 273 (1953)
56. Bertani, G., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 65 (1953)
57. Bertani, G., *Records Genetics Soc. Am.*, **22**, 66 (1953)
58. Bertani, G., and Nice, S. J., *Bact. Proc.*, 39 (1953)
59. Bertani, G., and Weigle, J. J., *J. Bact.*, **65**, 113 (1953)
60. Beth, K., *Z. Naturforsch.*, **8b**, 334 (1953)
61. Beth, K., *Z. Naturforsch.*, **8b**, 771 (1953)
62. Beumer, J., *Ann. inst. Pasteur*, **84**, 15 (1953)
63. Bevan, E. A., *Nature*, **171**, 576 (1953)
64. Bieser, S. M., and Davis, B. D., *Federation Proc.*, **12**, 436 (1953)
65. Birch, A. J., Donovan, F. W., and Moewus, F., *Nature*, **172**, 902 (1953)
66. Black, F. L., and Knight, C. A., *J. Biol. Chem.*, **202**, 51 (1953)
67. Bogorad, L., and Granick, S., *J. Biol. Chem.*, **202**, 793 (1953)
68. Bogorad, L., and Granick, S., *Proc. Natl. Acad. U. S.*, **39**, 1176 (1953)
69. Bonifas, V., and Novel, E., *Schweiz. Z. allgem. Pathol. u. Bakt.*, **15**, 391 (1952)
70. Brachet, J., and Szafarz, D., *Biochim. et Biophys. Acta*, **12**, 588 (1953)
71. Braun, W., *Bacterial Genetics* (W. B. Saunders Co., Philadelphia, Penna., 238 pp., 1953)
72. Bresch, C., *Ann. inst. Pasteur*, **84**, 157 (1953)
73. Brooke, M. S., and Magasanik, B., *Bact. Proc.*, **87** (1953)
74. Broquist, H. P., Kohler, R. A., Hutchison, D. J., and Burchenal, J. H., *J. Biol. Chem.*, **202**, 59 (1953)
75. Brown, G. L., and Watson, M., *Nature*, **172**, 339 (1953)
76. Brown, W., and Wood, R. K. S., *Adaptation in Microorganisms*, Chap. 15 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
77. Bruner, D. W., *J. Bact.*, **65**, 222 (1953)
77a. Bryson, V., Deiches, H., and Szybalski, W., *Am. Rev. Tuberc.*, **68**, 631 (1953)
78. Burnet, F. M., Fraser, K. B., and Lind, P. E., *Nature*, **171**, 163 (1953)
79. Burnet, F. M., and Lind, P. E., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 21 (1953)
80. Burnett, J. H., *New Phytologist*, **52**, 58 (1953)
81. Burnett, J. H., *New Phytologist*, **52**, 86 (1953)
82. Butler, G., *Can. J. Med. Sci.*, **31**, 222 (1953)
83. Cabelli, V. J., and Pickett, M. J., *J. Bact.*, **66**, 443 (1953)
84. Campbell, A. M., and Spiegelman, S., *Bact. Proc.*, 91 (1953)
85. Cantino, E. C., *Trans. N. Y. Acad. Sci.*, **15**, 159 (1953)
86. Cantino, E. C., and Hyatt, M. T., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **19**, 25 (1953)
87. Catlin, B. W., *J. Bact.*, **65**, 413 (1953)
88. Catlin, B. W., *J. Bact.*, **65**, 422 (1953)
88a. Cavalli, L. L., *Boll. ist. sieroterap. milan.*, **29**, 1 (1950)
89. Cavalli, L. L., Lederberg, J., and Lederberg, E. M., *J. Gen. Microbiol.*, **8**, 89 (1953)
90. Cavalli, L. L., and Maccacaro, G. A., *Heredity*, **6**, 311 (1952)
91. Cavalli-Sforza, L. L., and Lederberg, J., Symposium on Growth Inhibition and Chemotherapy, *Rept. Proc. 6th. Intern. Congr. Microbiol.*, 108 (Rome, Italy, 1953)
92. Chao, P. K., *Proc. Natl. Acad. Sci. U. S.*, **39**, 103 (1953)

93. Chargaff, E., Crampton, C. F., and Lipshitz, R., *Nature*, **172,** 289 (1953)
94. Chovnick, A., and Fox, A. S., *Am. Naturalist*, **87,** 263 (1953)
95. Clark, B. J., *J. Gen. Microbiol.*, **8,** 45 (1953)
96. Clegg, L. F. L., and Jacobs, S. E., *Adaptation in Microorganisms*, Chap. 14 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
97. Cohen, S. S., *Pediatrics*, **11, 89** (1953)
98. Cohen, S. S., *J. Biol. Chem.*, **201,** 71 (1953)
99. Cohen, S. S., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 221 (1953)
100. Cohen, S. S., and Roth, R., *J. Bact.*, **65,** 490 (1953)
101. Cohen-Bazire, G., and Jolit, M., *Ann. inst. Pasteur*, **84,** 937 (1953)
102. Cohn, M., Cohen, G. N., and Monod, J., *Compt. rend.*, **236,** 746 (1953)
103. Cohn, M., and Monod, J., *Adaptation in Microorganisms*, Chap. 6 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
104. Cohn, M., Monod, J., Pollock, M. R., Spiegelman, S., and Stanier, R. Y., *Nature*, **172,** 1096 (1953)
105. Cohn, M., and Torriani, A. M., *Biochim. et Biophys. Acta*, **10,** 280 (1953)
106. Davies, R., and Gale, E. F., Eds., *Adaptation in Microorganisms* (Cambridge University Press, Cambridge, England, 339 pp., 1953)
107. Davis, B. D., *Proc. Natl. Acad. Sci. U. S.*, **39,** 363 (1953)
108. Davis, B. D., *Science*, **118, 250** (1953)
109. Davis, B. D., Symposium on Microbial Metabolism, *Rept. Proc. 6th Intern. Congr. Microbiol.*, 23 (Rome, Italy, 1953)
110. Davis, B. D., and Mingioli, E. S., *J. Bact.*, **66,** 129 (1953)
111. Davis, B. D., and Weiss, U., *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmakol.*, **220,** 1 (1953)
112. Dean, A. C. R., and Hinshelwood, C., *Adaptation in Microorganisms*, Chap. 2 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
113. DeMars, R. I., *Nature*, **172,** 964 (1953)
114. DeMars, R. I., *Bact. Proc.*, **42** (1953)
115. DeMars, R. I., Luria, S. E., Fisher, H., and Levinthal, C., *Ann. inst. Pasteur*, **84,** 113 (1953)
116. Demerec, M., and Cahn, E., *J. Bact.*, **65,** 27 (1953)
117. DeSerres, F. J., and Giles, N. H., *Records Genetics Soc. Am.*, **22,** 71 (1953)
118. Dewey, D. L., and Work, E., *Nature*, **169,** 533 (1952)
119. Dianzani, M. U., *Exptl. Cell Research*, **5,** 311 (1953)
120. Dienes, L., *Proc. Soc. Exptl. Biol. Med.*, **83,** 579 (1953)
121. Dienes, L., *J. Bact.*, **66,** 274 (1953)
122. Doermann, A. H., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 3 (1953)
123. Doermann, A. H., and Hill, M. B., *Genetics*, **38,** 79 (1953)
124. Dorner, R. W., and Knight, C. A., *J. Biol. Chem.*, **205,** 959 (1953)
125. Dondero, N. C., and Zelle, M. R., *Science*, **118,** 34 (1953)
126. Doudney, C. O., and Wagner, R. P., *Proc. Natl. Acad. Sci. U. S.*, **39,** 1043 (1953)
127. Dukes, C. D., and Dukes, A. M., *Bact. Proc.*, 64 (1953)
128. Dulbecco, R., and Vogt, M., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 273 (1953)

129. Duraiswami, S., Sreepathi Roa, S. K., and Subramanian, M. K., *Experientia*, **9**, 293 (1953)
130. Dusi, H., *Compt. rend.*, **237**, 1795 (1953)
131. Eagle, H., Symposium on Growth Inhibition and Chemotherapy, 3, *Rept. Proc. 6th Intern. Congr. Microbiol.* (Rome, Italy, 1953)
132. Eddy, A. A., and Hinshelwood, C., *Proc. Roy. Soc.* (*London*), [B]**141**, 118 (1953)
133. Eddy, A. A., *Proc. Roy. Soc.* (*London*), [B]**141**, 126 (1953)
134. Edward, D. G., *J. Gen. Microbiol.*, **8**, 256 (1953)
135. Elion, G. B., Singer, S., and Hitchings, G. H., *J. Biol. Chem.*, **204**, 35 (1953)
135a. Elion, G. B., Singer, S., Hitchings, G. H., Balis, M. E., and Bosworth-Brown, G., *J. Biol. Chem.*, **202**, 647 (1953)
135b. Elion, G. B., Vanderwerff, H., Hitchings, G. H., Balis, M. E., Levin, D. H., and Bosworth-Brown, G., *J. Biol. Chem.*, **200**, 7 (1953)
136. Ephrussi, B., *Nucleo-Cytoplasmic Relations in Microorganisms* (Clarendon Press, Oxford, England, 127 pp., 1953)
137. Ephrussi, B., Leupold, U., Watson, J. D., and Weigle, J. J., *Nature*, **171**, 701 (1953)
138. Epstein, H. T., *Nature*, **171**, 394 (1953)
139. Evans, E. A., Jr., *Ann. inst. Pasteur*, **84**, 129 (1953)
140. Fernandez, B., Haas, F. L., and Wyss, O., *Proc. Natl. Acad. Sci. U. S.*, **39**, 1052 (1953)
140a. Fildes, P., and Van Heyningen, W. E., *The Nature of Virus Multiplication* (Cambridge University Press, Cambridge, England, 320 pp., 1953)
141. Finland, M., Wilcox, C., Wright, S. S., and Purcell, E. M., *Proc. Soc. Exptl. Biol. Med.*, **81**, 725 (1953)
142. Foley, G. E., and Watson, P. L., *Proc. Soc. Exptl. Med.*, **83**, 742 (1953)
143. Fouace, J., and Lutz, A., *Ann. inst. Pasteur*, **85**, 387 (1953)
144. Franklin, R. M., Friedman, M., and Setlow, R. B., *Arch. Biochem. and Biophys.*, **44**, 259 (1953)
145. Franklin, R. E., and Gosling, R. G., *Nature*, **172**, 156 (1953)
146. Franklin, R. E., and Gosling, R. G., *Nature*, **171**, 740 (1953)
147. Fraser, D., and Dulbecco, R., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 15 (1953)
148. Fraser, D., and Jerrel, E. A., *J. Biol. Chem.*, **205**, 291 (1953)
149. Fraser, D., and Williams, R. C., *Proc. Natl. Acad. Sci. U. S.*, **39**, 750 (1953)
150. Fredericq, P., *Ann. inst. Pasteur*, **84**, 294 (1953)
151. Friedman, M., and Cowles, P. B., *J. Bact.*, **66**, 379 (1953)
152. Friedman, S., and Gots, J. S., *J. Biol. Chem.*, **201**, 125 (1953)
153. Friedrich-Freksa, H., and Kaudewitz, F., *Z. Naturforsch.*, **8b**, 343 (1953)
154. Fries, N., and Aschan, K., *Svensk Botan. Tidskr.*, **46**, 429 (1952)
155. Gaden, E. L., and Henley, E. J., *J. Bact.*, **65**, 727 (1953)
156. Gandelman, B., Zamenhof, S., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 399 (1952)
157. Garber, E. D., and Hackett, A. J., *Nature* **173**, 88 (1954)
158. Garber, E. D., Noble, K., and Carouso, N., *J. Bact.*, **65**, 485 (1953)
159. Garnjobst, L., *Am. J. Botany*, **40**, 607 (1953)
160. Geckler, R. P., and Kimball, R. F., *Records Genetics Soc. Am.*, **22**, 73 (1953)
161. Giles, N. H., *Trans. N. Y. Acad. Sci.*, **15**, 251 (1953)
162. Giles, N. H., and Partridge, C. W. H., *Proc. Natl. Acad. Sci. U. S.*, **39**, 479 (1953)

163. Goebel, W. F., and Jesaitis, M. A., *Ann. inst. Pasteur*, **84,** 66 (1953)
164. Gots, J. S., and Hunt, G. R., Jr., *J. Bact.*, **66,** 353 (1953)
164a. Gowen, J. W., Stadler, J., Plough, H. H., and Miller, H. N., *Genetics*, **38,** 531 (1953)
165. Graham, A. F., *Ann. inst. Pasteur*, **84,** 90 (1953)
166. Graham, A. F., *Can. J. Med. Sci.*, **31,** 279 (1953)
167. Granick, S., Bogorad, L., and Jaffe, H., *J. Biol. Chem.*, **202,** 801 (1953)
168. Green, M., Alexander, M., and Wilson, P. W., *J. Bact.*, **66,** 623 (1953)
169. Grell, K. G., *Arch. Protistenk.*, **98,** 287 (1953)
170. Grell, K. G., *Arch. Protistenk.*, **98,** 466 (1953)
171. Grell, K. G., *Arch. Protistenk.*, **99,** 1 (1953)
172. Groman, N. B., *Bact. Proc.*, 41 (1953)
173. Groman, N. B., *Science*, **117,** 297 (1953)
174. Groman, N. B., *J. Bact.*, **66,** 184 (1953)
174a. Gunnison, J. B., and Jawetz, E., Symposium on Growth Inhibition and Chemotherapy, 19, *Rept. Proc. 6th Intern. Congr. Microbiol.*, 19 (Rome, Italy 1953)
175. Gunnison, J. B., Shevky, M. C., Bruff, J. A., Coleman, V. R., and Jawetz, E., *J. Bact.*, **66,** 150 (1953)
176. Haas, F. L., Wyss, O., Berger, H., and Stone, W. S., *J. Bact.*, **65,** 354 (1953)
177. Halvorson, H. O., and Spiegelman, S., *J. Bact.*, **65,** 601 (1953)
178. Halvorson, H. O., and Spiegelman, S., *J. Bact.*, **65,** 496 (1953)
179. Hannay, C. L., *Nature*, **172,** 1004 (1953)
179a. Harder, R., *Z. Botanik*, **19,** 337 (1927)
180. Harm, W., and Stein, W., *Z. Naturforsch.*, **8b,** 123 (1953)
181. Harm, W., and Stein, W., *Z. Naturforsch.*, **8b,** 729 (1953)
182. Hartelius, V., and Ditlevsen, E., *Compt. rend. trav. lab. Carlsberg Sér. physiol.*, **25,** 213 (1953)
183. Hartman, R. S., Eigelsbach, H. T., Bateman, J. B., Braun, W., Herring, R., and Rodgers, R. D., *Appl. Microbiol.*, **1,** 178 (1953)
184. Hartmann, M., *Arch. Protistenk.*, **98,** 125 (1953)
185. Haskins, F. A., Tissieres, A., Mitchell, H. K., and Mitchell, M. B., *J. Biol. Chem.*, **200,** 819 (1953)
186. Hassinger-Huizinga, H., *Arch. Protistenk.*, **98,** 90 (1953)
187. Hawthorne, D. C., *Bact. Proc.*, 30 (1953)
188. Hayes, W., *J. Gen. Microbiol.*, **8,** 72 (1953)
189. Hayes, W., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 75 (1953)
190. Hermann, E. C., Jr., and Steers, E., *J. Bact.*, **66,** 397 (1953)
191. Hershey, A. D., *Advances in Genetics*, **5,** 89 (1953)
192. Hershey, A. D., *Ann. inst. Pasteur*, **84,** 99 (1953)
193. Hershey, A. D., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 135 (1953)
194. Hershey, A. D., *J. Gen. Physiol.*, **37,** 1 (1953)
195. Hershey, A. D., Dixon, J., and Chase, M., *J. Gen. Physiol.*, **36,** 777 (1953)
196. Herzberg, K., and May, G., *Naturwissenschaften*, **40,** 367 (1953)
197. Hewitt, L. F., *J. Gen. Microbiol.*, **7,** 362 (1952)
198. Hewitt, L. F., *Adaptation in Microorganisms*, Chap. 12 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
199. Hirsch, H. M., *Biochim. et Biophys. Acta*, **9,** 674 (1952)
200. Hirsch, M. L., and Cohen, G. N., *Biochem. J.* (*London*), **53,** 25 (1953)

201. Hirsch, M. L., and Cohen, G. N., *Compt. rend.*, **236,** 2338 (1953)
201a. Hirsch, M. L., and Cohen, G. N., *Compt. rend.*, **236,** 1302 (1953)
202. Hirst, G. K., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 25 (1953)
203. Hirst, G. K., and Gotlieb, T., *J. Exptl. Med.*, **98,** 41 (1953)
204. Hirst, G. K., and Gotlieb, T., *J. Exptl. Med.*, **98,** 53 (1953)
205. Hofsten, B. von, Hofsten, A. von, and Fries, N., *Exptl. Cell Research*, **5,** 530 (1953)
206. Hollaender, A., and Stapleton, G. E., *Physiol. Revs.*, **33,** 77 (1953)
207. Holton, C. S., *Botan. Rev.*, **19,** 187 (1953)
208. Horowitz, N. H., and Fling, M., *Genetics*, **38,** 360 (1953)
209. Houtermans, T., *Z. Naturforsch.*, **8b,** 767 (1953)
210. Hughes, W. H., *J. Gen. Microbiol.*, **8,** 307 (1953)
211. Huybers, K., *Ann. inst. Pasteur*, **84,** 242 (1953)
212. Ionesco, H., *Compt. rend.*, **237,** 1794 (1953)
213. Jacob, F., *Ann. inst. Pasteur*, **83,** 671 (1952)
214. Jacob, F., *Ann. inst. Pasteur*, **84,** 254 (1953)
215. Jacob, F., Lwoff, A., Siminovitch, A., and Wollman, E., *Ann. inst. Pasteur*, **84,** 222 (1953)
216. Jacob, F., Siminovitch, L., and Wollman, E. L., *Ann. inst. Pasteur*, **84,** 313 (1953)
217. Jacob, F., and Wollman, E. L., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 101 (1953)
218. Jacob, F., Wollman, E., and Siminovitch, L., *Compt. rend.*, **236,** 544 (1953)
219. James, A. P., *Records Genetics Soc. Am.*, **22,** 80 (1953)
220. Jensen, J., and Thofern, E., *Z. Naturforsch.*, **8b,** 599 (1953)
221. Jensen, J., and Thofern, E., *Z. Naturforsch.*, **8b,** 604 (1953)
222. Jensen, J., and Thofern, E., *Z. Naturforsch.*, **8b,** 697 (1953)
223. Jesaitis, M. A., and Goebel, W. F., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 205 (1953)
224. Jesaitis, M. A., and Goebel, W. F., *Nature*, **172,** 622 (1953)
225. Jinks, J. L., *Nature*, **170,** 106 (1952)
226. Jinks, J. L., *Compt. rend. trav. lab. Carlsberg Sér. physiol.*, **25,** 252 (1953)
226a. Kahler, H., and Lloyd, B. J., *Biochim. et Biophys. Acta*, **10,** 355 (1953)
227. Kaplan, R. W., *Arch. Mikrobiol.*, **18,** 210 (1953)
228. Katagiri, M., and Sato, R., *Science*, **118,** 250 (1953)
229. Kaudewitz, F., *Z. Naturforsch.*, **8b,** 500 (1953)
230. Keosian, J., *Records Genetics Soc. Am.*, **22,** 83 (1953)
231. Kimball, R. F., and Gaither, N., *Records Genetics Soc. Am.*, **22,** 83 (1953)
232. Kimball, R. F., and Gaither, N., *Proc. Soc. Exptl. Biol. Med.*, **82,** 471 (1953)
233. Klein, D. T., and Klein, R. M., *J. Bact.*, **66,** 220 (1953)
234. Klein, M., and Schorr, S. E., *J. Bact.*, **65,** 454 (1953)
235. Koch, A. L., *Bact. Proc.*, 88 (1953)
236. Kolmark, G., *Hereditas*, **39,** 270 (1953)
237. Kolmark, G , and Giles, N. H., *Records Genetics Soc. Am.*, **22,** 84 (1953)
238. Kolmark, G., and Westergaard, M., *Hereditas*, **39,** 209 (1953)
239. Kozloff, L. M., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 209 (1953)
240. Kroger, E., *Z. Naturforsch.*, **8b,** 133 (1953)
241. Kubitschek, H. E., and Bendigkeit, H. E., *Bact. Proc.*, **54,** (1953)
242. Kuwana, H., *Cytologia (Tokyo)*, **18,** 235 (1953)
243. Labrum, E. L., *Proc. Natl. Acad. Sci. U. S.*, **39,** 280 (1953)

244. Labrum, E. L., *Proc. Natl. Acad. Sci. U. S.*, **39,** 1221 (1953)
245. Labrum, E. L., and Bunting, M. I., *J. Bact.*, **65,** 394 (1953)
246. Langendorff, H., Langendorff, M., and Sommermeyer, K., *Z. Naturforsch.*, **8b,** 117 (1953)
247. Lanni, F., and Lanni, Y., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 159 (1953)
247a. Lark, K. G., and Adams, M. H., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 171 (1953)
248. Latarjet, R., *Compt. rend.*, **235, 1701** (1952)
248a. Lederberg, J., *Physiol. Revs.*, **32,** 403 (1952)
249. Lederberg, J., and Edwards, P. R., *J. Immunol.*, **71,** 232 (1953)
250. Lederberg, E. M., and Lederberg, J., *Genetics*, **38,** 51 (1953)
251. Lederberg, J., and Tatum, E. L., *Science*, **118,** 169 (1953)
252. Lee, H. H., *Arch. Biochem. and Biophys.*, **47,** 438 (1953)
253. Leidy, G., Hahn, E., and Alexander, H. E., *J. Exptl. Med.*, **97,** 467 (1953)
254. Lein, J., Puglisi, T. A., and Lein, P. S., *Arch. Biochem. and Biophys.*, **45,** 434 (1953)
255. Lenert, T. F., and Hobby, G. L., *Bact. Proc.*, 69 (1953)
256. Leupold, U., and Horowitz, N. H., *Z. ind. Abstam. Vererbungsl.*, **84,** 306 (1952)
257. Levine, M., *Records Genetics Soc. Am.*, **22,** 85 (1953)
257a. Levine, M., *Evolution*, **7,** 366 (1953)
257b. Levine, M., *Genetics*, **38,** 561 (1953)
257c. Levine, S., Puck, T. T., and Sagik, B., *J. Exptl. Med.*, **98,** 521 (1953)
258. Levinthal, C., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 13 (1953)
259. Levinthal, C., and Fisher, H. W., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 29 (1953)
260. Levinthal, C., and Visconti, N., *Genetics*, **38,** 500 (1953)
261. Lewin, R. A., *J. Genetics*, **51,** 543 (1953)
262. Lieb, M., *J. Bact.*, **65,** 642 (1953)
263. Lieb, M., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 71 (1953)
264. Liebermeister, K., *Z. Naturforsch.*, **8b,** 757 (1953)
265. Lind, P. E., and Burnet, F. M., *Australian J. Exptl. Biol. Med. Sci.*, **31,** 361 (1953)
266. Lindegren, C. C., and Lindegren, G., *Genetica*, **26,** 430 (1953)
267. Lindegren, C. C., *J. Genetics*, **51,** 625 (1953)
268. Lindegren, C. C., Braham, J. E., and DeDios Calle, J., *Nature*, **172,** 800 (1953)
269. Lindegren, C. C., and Haddard, S. A., *Exptl. Cell Research*, **5,** 549 (1953)
270. Lindegren, C. C., and Lindegren, G., *Genetics*, **38,** 73 (1953)
271. Lindegren, C. C., and Lindegren, G., *Bact. Proc.*, 30 (1953)
272. Lindegren, C. C., and Pittman, D. D., *J. Gen. Microbiol.*, **9,** 494 (1953)
273. Lindenman, J., *Experientia*, **9,** 22 (1953)
274. Lucke, W. H., and Sarachek, A., *Nature*, **171,** 1014 (1953)
275. Luria, S. E., *General Virology* (John Wiley and Sons, Inc., New York, N. Y., 427 pp., 1953)
276. Luria, S. E., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 237 (1953)
277. Ludlum, B. G., *J. Hyg.*, **51,** 64 (1953)
278. Lwoff, A. E., *Bact. Revs.*, **17,** 269 (1953)
278a. Lwoff, A., *Ann. inst. Pasteur*, **84,** 1 (1953)
279. Lwoff, A., *Ann. inst. Pasteur*, **84,** 223 (1953)
280. Lwoff, A., Kaplan, A. S., and Ritz, E., *Compt. rend.*, **236,** 2126 (1953)

281. Maaløe, O., and Symonds, N., *J. Bact.*, **65**, 177 (1953)
282. Maas, W. K., and Vogel, H. J., *J. Bact.*, **65**, 388 (1953)
283. Magne, F., *Compt. rend.*, **236**, 1596 (1953)
284. Manten, A., and Rowley, D., *J. Gen. Microbiol.*, **9**, 226 (1953)
285. Manten, A., and Rowley, D., *Brit. J. Exptl. Pathol.*, **34**, 23 (1953)
286. Manten, A., and Wijngaarden, L. J., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **19**, 93 (1953)
287. Marcovich, H., *Ann. inst. Pasteur*, **85**, 199 (1953)
288. Markert, C. L., *Exptl. Cell Research*, **5**, 427 (1953)
289. Markham, R., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 141 (1953)
290. McMurtrie, E., and Berman, D. T., *Bact. Proc.*, 47 (1953)
290a. Mefferd, R. B., and Loefer, J. B., *Physiol. Zoöl.*, **26**, 241 (1953)
291. Merkel, J. R., and Nickerson, W. J., *Proc. Natl. Acad. Sci. U. S.*, **39**, 1008 (1953)
292. Merkel, J. R., and Steers, E., *J. Bact.*, **66**, 389 (1953)
293. Minck, R., *Compt. rend.*, **236**, 250 (1953)
294. Mitchell, H. K., Symposium on Nutrition and Growth factors, *Rept. Proc. 6th Intern. Congr. Microbiol.*, 75 (Rome, Italy, 1953)
295. Mitchell, M. B., Mitchell, H. K., and Tissieres, A., *Proc. Natl. Acad. Sci. U. S.*, **39**, 606 (1953)
296. Mitchison, D. A., *J. Gen. Microbiol.*, **8**, 168 (1953)
297. Mitchison, D. A., *Adaptation in Microorganisms*, Chap. 11 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
298. Mittler, S., and Laverty, J. A., *Nature*, **171**, 793 (1953)
299. Monod, J., and Cohen-Bazire, G., *Compt. rend.*, **236**, 417 (1953)
300. Monod, J., and Cohen-Bazire, G., *Compt. rend.*, **236**, 530 (1953)
300a. Monod, J., and Cohn, M., Symposium on Microbial Metabolism, *Rept. Proc. 6th Intern. Congr. Microbiol.*, 43 (Rome, Italy, 1953)
301. Monnot, F., *Compt. rend.*, **236**, 2263 (1953)
302. Monnot, F., *Compt. rend.*, **236**, 2330 (1953)
302a. Morowitz, H. J., *Arch. Biochem. and Biophys.*, **47**, 325 (1953)
303. Mortimer, R. K., and Tobias, C. A., *Science*, **118**, 517 (1953)
304. Mudd, S., Hillier, J., Beutner, E. H., and Hartman, P. E., *Biochim. et Biophys. Acta*, **10**, 153 (1953)
305. Mundkur, B. D., *Nature*, **171**, 793 (1953)
306. Mundkur, B. D., *Experientia*, **9**, 373 (1953)
306a. Murphy, J. S., *J. Exptl. Med.*, **96**, 581 (1953)
307. Murphy, J. S., *J. Exptl. Med.*, **98**, 657 (1953)
308. Murray, R. G. E., and Whitfield, J. F., *J. Bact.*, **65**, 715 (1953)
309. Mutsaars, W., *Ann. inst. Pasteur*, **85**, 1 (1953)
310. Nanney, D. L., *Proc. Natl. Acad. Sci. U. S.*, **39**, 113 (1953)
311. Nanney, D. L., *Biol. Bull.*, **105**, 133 (1953)
312. Nanney, D. L., and Caughey, P. A., *Proc. Natl. Acad. Sci. U. S.*, **39**, 1057 (1953)
313. Nason, A., Kaplan, N. O., and Oldewurtel, H. A., *J. Biol. Chem.*, **201**, 435 (1953)
314. Nelson, E. V., and Shapiro, S. K., *Bact. Proc.*, 87 (1953)
315. Newcombe, H. B., *Genetics*, **38**, 134 (1953)
315a. Newcombe, H. B., *J. Gen. Microbiol.*, **9**, 30 (1953)

316. Newmeyer, D., and Tatum, E. L., *Am. J. Botany*, **40**, 393 (1953)
317. Nicolle, P., Jude, A., and Diverneau, G., *Ann. inst. Pasteur*, **84**, 27 (1953)
318. Norman, A., *J. Bact.*, **65**, 151 (1953)
319. Northrop, J. H., *Biochim. et Biophys. Acta*, **12**, 41 (1953)
320. Northrop, J. H., *J. Gen. Physiol.*, **36**, 581 (1953)
321. Nybom, N., *Hereditas*, **39**, 317 (1953)
322. Ogur, M., Lindegren, G., and Lindegren, C. C., *Records Genetics Soc. Am.*, **22**, 90 (1953)
323. Olive, L. S., *Botan. Rev.*, **19**, 439 (1953)
324. Palleroni, N. J., and Lindegren, C. C., *J. Bact.*, **65**, 122 (1953)
325. Papazian, H. P., *Genetics*, **37**, 175 (1952)
326. Pardee, A. B., and Williams, I., *Ann. inst. Pasteur*, **84**, 147 (1953)
327. Parker, R. B., Elliker, P. R., and Smith, V. N., *Bact. Proc.*, **78** (1953)
328. Partridge, C. W., and Giles, N. H., *Am. J. Botany*, **40**, 778 (1953)
329. Perkins, D. D., *Genetics*, **38**, 187 (1953)
330. Pittenger, R. C., and McCoy, E., *J. Bact.*, **65**, 56 (1953)
331. Pittenger, T. H., *Records Genetics Soc. Am.*, **22**, 93 (1953)
332. Pittman, D. D., and Lindegren, C. C., *Records Genetics Soc. Am.*, **22**, 93 (1953)
333. Pollock, M. R., *Adaptation in Microorganisms*, Chap. 7 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
334. Pollock, M. R., and Torriani, A. M., *Compt. rend.*, **237**, 276 (1953)
335. Pomper, S., *J. Bact.*, **65**, 666 (1953)
336. Pomper, S., and McKee, D. W., *Science*, **117**, 62 (1953)
337. Pomper, S., and McKee, D. W., *Science*, **117**, 455 (1953)
337a. Pontecorvo, G., *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 193 (1946)
338. Pontecorvo, G., *Advances in Genetics*, **5**, 141 (1953)
339. Pontecorvo, G., Tarr Gloor, E., and Forbes, E., *J. Genetics*, **52**, 226 (1954)
340. Pontecorvo, G., Roper, J. A., and Forbes, E., *J. Gen. Microbiol.*, **8**, 198 (1953)
341. Pontecorvo, G., and Sermonti, G., *Nature*, **172**, 126 (1953)
342. Preer, J. R., and Siegel, R. W., *Records Genetics Soc. Am.*, **22**, 94 (1953)
343. Preer, J. R., Siegel, R. W., and Stark, P. S., *Proc. Natl. Acad. Sci. U. S.*, **39**, 1228 (1953)
344. Preer, J. R., and Stark, P. S., *Exptl. Cell Research*, **5**, 478 (1953)
345. Prout, T., Huebschman, C., Levene, H., and Ryan, F. J., *Genetics*, **38**, 518 (1953)
350. Puck, T. T., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 149 (1953)
350a. Puck, T. T., and Sagik, B., *J. Exptl. Med.*, **97**, 807 (1953)
351. Purcell, E. M., Wright, S. S., and Finland, M., *Proc. Soc. Exptl. Biol. Med.*, **82**, 124 (1953)
352. Raettig, H., *Zentr. Bakt. Parasitenk., Abt. I, Orig.*, **159**, 240 (1953)
353. Raper, J. R., *Quart. Rev. Biol.*, **28**, 233 (1953)
354. Raper, K. B., and Fennell, D. I., *J. Elisha Mitchell Sci. Soc.*, **69**, 1 (1953)
355. Raut, C., *Exptl. Cell Research*, **4**, 295 (1953)
356. Ravin, A. W., *Adaptation in Microorganisms*, Chap. 3 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
357. Reisner, A. R., Barratt, R. W., and Newmeyer, D., *Records Genetics Soc. Am.*, **22**, 95 (1953)
358. Rizet, G., *Rev. cytol. biol. végétales*, **13**, 51 (1952)

359. Rizet, G., *Compt. rend.*, **237,** 1106 (1953)
360. Rizet, G., *Compt. rend.*, **237,** 666 (1953)
361. Rizet, G., and Esser, K., *Compt. rend.*, **237,** 760 (1953)
362. Robbins, W. J., Hervey, A., and Stebbins, M. E., *Ann. N. Y. Acad. Sci.*, **56,** 818 (1953)
363. Roman, H., and Sands, S. M., *Proc. Natl. Acad. Sci. U. S.*, **39,** 171 (1953)
364. Roper, J. A., *Experientia*, **8,** 14 (1952)
365. Rosanoff, E. I., and Sevag, M. G., *Antibiotics & Chemotherapy*, **3,** 495 (1953)
366. Rotman, B., and Spiegelman, S., *J. Bact.*, **66,** 492 (1953)
367. Rotman, B., and Spiegelman, S., *Bact. Proc.*, 92 (1953)
368. Rowley, D., *Nature*, **171,** 80 (1953)
369. Rubin, B. A., Perry, M. F. C., and Thanassi, F. Z., *Bact. Proc.*, 32 (1953)
370. Rudman, D., and Meister, A., *J. Biol. Chem.*, **200,** 591 (1953)
371. Ryan, F. J., *Nature*, **171,** 400 (1953)
372. Ryan, F. J., *Am. Naturalist*, **87,** 383 (1953)
373. Ryan, F. J., Kunin, C., Ballentine, R., and Maas, W., *J. Bact.*, **65,** 434 (1953)
374. Sager, R., and Granick, S., *Ann. N. Y. Acad. Sci.*, **56,** 831 (1953)
375. Sarachek, A., and Lucke, W. H., *Experientia*, **9,** 374 (1953)
376. Sarachek, A., and Lucke, W. H., *Arch. Biochem. and Biophys.*, **44,** 271 (1953)
377. Sarachek, A., and Townsend, G. F., *Science*, **117,** 31 (1953)
378. Saz, A. K., and Eagle, H., *J. Bact.*, **66,** 347 (1953)
379. Schaeffer, P., *Arch. Biochem. and Biophys.*, **47,** 359 (1953)
380. Schramm, G., and Brannitzer, G., *Z. Naturforsch.*, **8b,** 61 (1953)
381. Seshachar, B. R., and Dass, C. M. S., *Exptl. Cell Research*, **5,** 248 (1953)
382. Sevag, M. G., and Rosanoff, E. I., *Arch. Biochem. and Biophys.*, **45,** 233 (1953)
383. Shapiro, S. K., *J. Bact.*, **65,** 310 (1953)
383a. Siegel, R. W., *Genetics*, **38,** 550 (1953)
384. Siegel, A. L., Rappaport, I., and Wildman, S. G., *Records Genetics Soc. Am.*, **22,** 101 (1953)
385. Silver, W. S., and McElroy, W. D., *Federation Proc.*, **12,** 268 (1953)
386. Siminovitch, L., *Ann. inst. Pasteur*, **84,** 265 (1953)
387. Singleton, J. R., *Am. J. Botany*, **40,** 124 (1953)
388. Slonimski, P. P., *Recherches sur la Formation des Ensymes Respiratoires chez la Levure* (Editions Desoer, Liége, Belgium, 203 pp., 1953)
389. Slonimski, P. P., *Adaptation in Microorganisms*, Chap. 4 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
390. Slonimski, P. P., and Hirsch, H. M., *Compt. rend.*, **235,** 741 (1952)
391. Smith, W. H., *J. Gen. Physiol.*, **8,** 116 (1953)
392. Smolens, J., and Vogt, A. B., *J. Bact.*, **66,** 140 (1953)
393. Spiegelman, S., *Science*, **116,** 510 (1952)
394. Spiegelman, S., and Halvorson, H. O., *Adaptation in Microorganisms*, Chap. 5 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
395. Srb, A. M., *Records Genetics Soc. Am.*, **22,** 104 (1953)
396. Stanier, R. Y., *Adaptation in Microorganisms*, Chap. 1 (Davies, R., and Gale, E. F., Eds., Cambridge University Press, Cambridge, England, 339 pp., 1953)
397. Stapleton, G. E., Billen, D., and Hollaender, A., *J. Cellular Comp. Physiol.*, **41,** 345 (1953)

398. Stein, W., and Harm, W., *Naturwissenschaften*, **39**, 113 (1952)
399. Stein, W., and Harm, W., *Naturwissenschaften*, **39**, 383 (1952)
400. Stein, W., and Harm, W., *Z. Naturforsch.*, **8b**, 742 (1953)
401. Stent, G. S., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 255 (1953)
402. Stent, G. S., *Proc. Natl. Acad. Sci. U. S.*, **39**, 1234 (1953)
403. Stent, G. S., and Maaløe, O., *Biochim. et Biophys. Acta*, **10**, 55 (1953)
404. Stern, C., *Genetics*, **21**, 625 (1936)
405. Stevens, C. M., and Mylroie, A., *Nature*, **171**, 179 (1953)
406. Stevens, C. M., and Mylroie, A., *Am. J. Botany*, **40**, 424 (1953)
407. Stich, H., *Z. Naturforschung*, **8b**, 36 (1953)
408. Stich, H., and Hämmerling, J., *Z. Naturforschung*, **8b**, 329 (1953)
409. Stock, C. C., Williamson, M., and Jacobson, W. E., *Ann. N. Y. Acad. Sci.*, **56**, 1081 (1953)
410. Stocker, B. A. D., Krauss, M. R., and MacLeod, C. M., *J. Pathol. Bact.*, **66**, 330 (1953)
411. Stocker, B. A. D., Zinder, N. D., and Lederberg, J. J., *J. Gen. Microbiol.*, **9**, 410 (1953)
412. Strauss, B. S., *Arch. Biochem. and Biophys.*, **44**, 200 (1953)
413. Strauss, B. S., *Records Genetics Soc. Am.*, **22**, 105 (1953)
414. Sussman, A. S., and Markert, C. L., *Arch. Biochem. and Biophys.*, **45**, 31 (1953)
415. Sussman, M., *Bact. Proc.*, **35** (1953)
416. Sussman, M., and Bradley, S. G., *J. Bact.*, **66**, 52 (1953)
417. Sussman, R. R., and Sussman, M., *Ann. N. Y. Acad. Sci.*, **56**, 949 (1952)
418. Szybalski, W., *Antibiotics & Chemotherapy*, **3**, 915 (1953)
419. Szybalski, W., *Antibiotics & Chemotherapy*, **3**, 1095 (1953)
420. Szybalski, W., and Bryson, V., *Am. Rev. Tuberc.*, **68**, 280 (1953)
421. Szybalski, W., and Bryson, V., *J. Bact.*, **66**, 468 (1953)
422. Terra, P., *Compt. rend.*, **236**, 115 (1953)
423. Terra, P., *Compt. rend.*, **236**, 1438 (1953)
424. Tissieres, A., Mitchell, H. K., and Haskins, F. A., *J. Biol. Chem.*, **205**, 423 (1953)
425. Umbarger, H. E., *J. Bact.*, **66**, 328 (1953)
426. Umbarger, H. E., *J. Bact.*, **65**, 203 (1953)
426a. Umbarger, H. E., and Adelberg, E., *J. Biol. Chem.*, **192**, 483 (1951)
427. Utiger, H., *Naturwissenschaften*, **40**, 292 (1953)
429. Van Wagtendonk, W. J., and van Tijn, B., *Exptl. Cell Research*, **5**, 1 (1953)
430. Vendrely, R., and Tulasne, R., *Nature*, **171**, 262 (1953)
431. Visconti, N., *Ann. inst. Pasteur*, **84**, 180 (1953)
432. Visconti, N., *J. Bact.*, **66**, 247 (1953)
433. Visconti, N., and Delbrück, M., *Genetics*, **38**, 5 (1953)
434. Visconti, N., and Garen, A., *Proc. Natl. Acad. Sci. U. S.*, **39**, 620 (1953)
435. Vogel, H. J., *Records Genetics Soc. Am.*, **22**, 107 (1953)
436. Vogel, H. J., *Proc. Natl. Acad. Sci. U. S.*, **39**, 578 (1953)
437. Vogesland, T. M., *Acta Pathol. Microbiol. Scand.*, **33**, 301 (1953)
438. von Prittwitz und Gaffron, J., *Naturwissenschaften*, **40**, 590 (1953)
439. Wahl, R., *Ann. inst. Pasteur*, **84**, 51 (1953)
440. Wainwright, S. D., *Records Genetics Soc. Am.*, **22**, 108 (1953)
441. Wainwright, S. D., and Mullaney, J., *Experientia*, **9**, 376 (1953)
441a. Warren, K. B., Ed., *Cold Spring Harbor Symposia Quant. Biol.*, **18** (The Biological Laboratory, Cold Spring Harbor, Long Island, N. Y., 301 pp. 1953)
442. Wasserman, M. M., and Seligman, E., *J. Bact.*, **66**, 119 (1953)

443. Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737 (1953)
444. Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 964 (1953)
445. Watson, J. D., and Crick, F. H. C., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 123 (1953)
446. Watson, J. D., and Hayes, W., *Proc. Natl. Acad. Sci. U. S.*, **39**, 416 (1953)
447. Watson, J. D., and Maaløe, O., *Biochim. et Biophys. Acta*, **10**, 432 (1953)
448. Weidel, W., *Z. Naturforsch.*, **7b**, 145 (1952)
449. Weidel, W., *Ann. inst. Pasteur*, **84**, 60 (1953)
450. Weidel, W., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 155 (1953)
451. Weigle, J. J., *Proc. Natl. Acad. Sci. U. S.*, **39**, 628 (1953)
452. Weigle, J. J., and Bertani, G., *Ann. inst. Pasteur*, **84**, 175 (1953)
453. Weigle, J. J., and Dulbecco, R., *Experientia*, **9**, 372 (1953)
454. Welsch, M., Cavallo, G., and Cantelmo, P., *Rev. belge pathol. et méd. Exptl.*, **22**, 241 (1953)
455. Wilkins, M. H. F., Stokes, A. R., and Wilson, H. R., *Nature*, **171**, 738 (1953)
456. Winge, O., and Roberts, C., *Compt. rend. trav. lab. Carlsberg, Sér. physiol.*, **25**, 241 (1953)
457. Witkin, E., *Proc. Natl. Acad. Sci. U. S.*, **30**, 427 (1953)
458. Wolinsky, E., and Steenken, W., Jr., *J. Bact.*, **66**, 229 (1953)
459. Wollman, E. L., *Ann. inst. Pasteur*, **84**, 281 (1953)
460. Wood, T. H., *Proc. Soc. Exptl. Biol. Med.*, **84**, 446 (1953)
461. Wright, B. E., *Compt. rend. trav. lab. Carlsberg, Sér. physiol.*, **25**, 173 (1953)
462. Wright, B. E., *Nature*, **172**, 308 (1953)
463. Wright, B. E., *J. Bact.*, **66**, 407 (1953)
464. Wyatt, G. R., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 133 (1953)
465. Wyatt, G. R., and Cohen, S. S., *Ann. inst. Pasteur*, **84**, 143 (1953)
466. Wyss, O., and Schaiberger, G. E., *J. Bact.*, **66**, 49 (1953)
467. Yaniv, H., Avi-Dor, Y., and Olitzki, A. L., *Experentia*, **9**, 23 (1953)
468. Yaniv, H., and Avi-Dor, Y., *J. Bact.*, **66**, 6 (1953)
469. Yaniv, H., and Davis, B. D., *J. Bact.*, **66**, 238 (1953)
470. Yanofsky, C., *Records Genetics Soc. Am.*, **22**, 112 (1953)
471. Ycas, M., and Starr, T. J., *J. Bact.*, **65**, 83 (1953)
472. Yudkin, J., *Nature*, **171**, 541 (1953)
473. Zalokar, M., *Arch. Biochem. and Biophys.*, **44**, 330 (1953)
474. Zamenhof, S., Alexander, H. E., and Leidy, G., *Can. J. Med. Sci.*, **31**, 252 (1953)
475. Zamenhof, S., Alexander, H. E., and Leidy, G., *J. Exptl. Med.*, **98**, 373 (1953)
476. Zamenhof, S., Leidy, G., Alexander, H. E., Fitzgerald, P. L., and Chargaff, E., *Arch. Biochem. and Biophys.*, **40**, 50 (1952)
477. Zamenhof, S., Leidy, G., Fitzgerald, P. L., Alexander, H. E., and Chargaff, E., *J. Biol. Chem.*, **203**, 695 (1953)
478. Zickler, H., *Arch. Protistenk.*, **98**, 1 (1953)
479. Ziegler, A. W., *Am. J. Botany*, **40**, 60 (1953)
480. Zinder, N. D., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 261 (1953)
481. Zirkle, R. E., and Tobias, C. A., *Arch. Biochem. and Biophys.*, **47**, 282 (1953)

BACTERIAL VIRUSES[1]

With Particular Reference to the Synthesis of

By E. A. Evans, Jr.

Department of Biochemistry, University of Chicago, Chicago, Illinois

The last review of this subject (1) included literature up to April 1952, and the present report is concerned primarily with papers published between this date and December 1953. During this period there have been four international meetings devoted partly or wholly to the subject of bacterial viruses, for which the proceedings have been published (2 to 5). Specific references will be made to various papers in these proceedings, but many of those not cited contain detailed information of great value to investigators interested in this field. A number of reviews, monographs, and text books have also appeared (6 to 12).

Coliphages

Although our knowledge of other bacterial host-virus systems is increasing, much of our present knowledge concerning the mechanism of phage reproduction is derived from a study of the "T" series of coliphages. Studies of the morphology of these phages and of infected bacterial cells by electron microscopy and other techniques continue to appear (13 to 22). Although there is some variation in the dimensions given by various workers (15, 18), there is general agreement that the particles are sperm-like in shape with tails of varying sizes. In view of the fact that the phage tail apparently constitutes the point of attachment to the sensitive bacterial host (23), it is of interest that T3 and T7, which were formerly thought to be spherical, are now shown to possess stubby tails. Chemically, the coliphages are composed of nucleic acid of the desoxyribonucleic acid type and protein. Fraser & Jerrel (24) have determined the amino acid content of T3 bacteriophage. They find that the amino acid content of the virus remains the same under a variety of conditions, including alteration of the carbon and nitrogen sources of the host bacterium. The amino acid distribution for T3 is different, but qualitatively similar to that previously described for T4. The presence of small amounts of ribonucleic acid and lipid material has been reported, although not in every case (25). For the T-even phages, the DNA[1] is apparently confined to the head, with an outer coating and tail composed of protein. In the intact state, the DNA of the virus particle is resistant to desoxyribonuclease. However, if concentrated solutions of the even-numbered viruses in sodium chloride or other solutes are diluted rapidly with distilled water, the DNA is released into the solution in a form which is sensitive to desoxyribonuclease action and which appears to be unorganized as seen by

[1] The following abbreviations have been used in this chapter: DNA (desoxyribonucleic acid); HMC (hydroxymethylcytosine); RNA (ribonucleic acid).

the electron microscope (26). By this method of "osmotic shock" the bulk or all of the protein can be obtained in the form of "ghost" preparations. With phage T2, Lanni & Lanni (27) have demonstrated the presence of two antigens in such ghost preparations, one associated with the head, and the

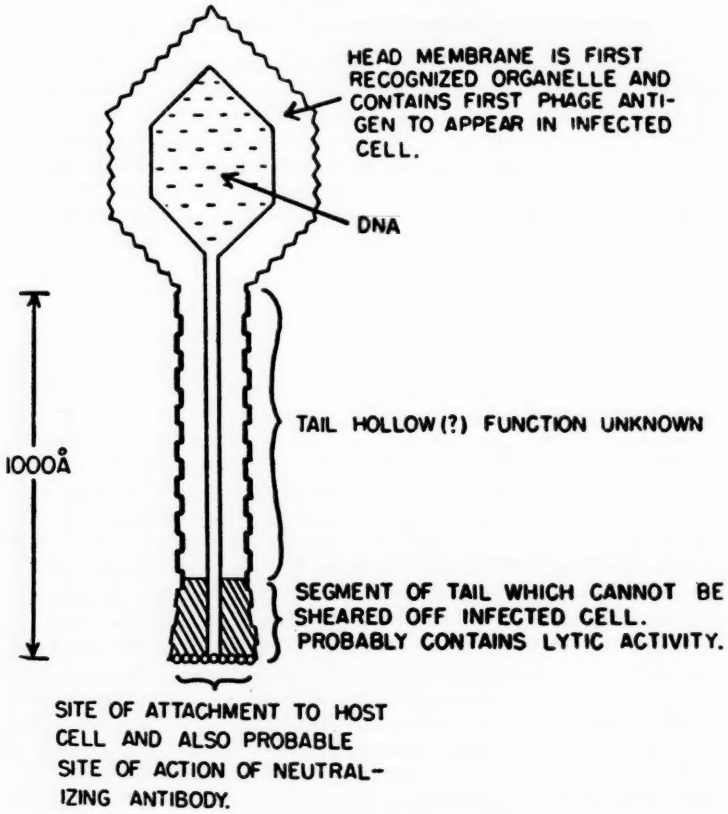

HEAD MEMBRANE IS FIRST RECOGNIZED ORGANELLE AND CONTAINS FIRST PHAGE ANTIGEN TO APPEAR IN INFECTED CELL.
DNA
1000Å
TAIL HOLLOW (?) FUNCTION UNKNOWN
SEGMENT OF TAIL WHICH CANNOT BE SHEARED OFF INFECTED CELL. PROBABLY CONTAINS LYTIC ACTIVITY.
SITE OF ATTACHMENT TO HOST CELL AND ALSO PROBABLE SITE OF ACTION OF NEUTRALIZING ANTIBODY.

FIG. 1. Schematic diagram of the T-even bacteriophage particle. [From Anderson, T. F. (21)].

other with the tail, and it is generally believed that the antigenic properties of the phage particle are a property of the protein coat. It should be pointed out that susceptibility to osmotic shock is exhibited only by the even-numbered phages. Figure 1 from Anderson (21) summarizes the combined studies of many workers on the functional anatomy of the T-even particles.

Other Phages

A number of new virus-host systems have been described: for example, five phages for *Bacillus megaterium* (28, 29), 12 for *Salmonella typhosa* (30), and a number acting on *B. mycoides* N (31, 32, 33) and other organisms (34 to 38). Two phages acting on *Clostridium perfringens* are now known (39), and it has also been shown that the coliphages can be synthesized anerobically (40). In view of the fact that much of our information regarding phage reproduction has been obtained with the T coliphages, it is highly desirable to have other virus-host systems which can be used for testing general conclusions. A general basis for the classification of bacterial viruses has been set forth by Adams (41).

Host-Virus Relationships

In terms of their behavior toward suitable bacterial hosts, phages have been classified into two categories, virulent and temperate. These terms imply two types of host-virus relationships, the lytic and the lysogenic, respectively, and it will be convenient to consider our information in regard to them separately.

Virulent phages.—When virulent viral particles are adsorbed by a sensitive host, the infecting particle is fragmented or split and it appears that only the nucleic acid of the virus (i.e., its DNA) participates in the further stages of virus reproduction, although a small amount of parent protein may also be involved. In any event, it is impossible to demonstrate for a time the presence of infectious virus particles inside the cell, but shortly they appear in increasing numbers until the cell lyses, with the liberation of many particles identical with the original parent. Most of our detailed information concerning phage reproduction is concerned with this lytic process.

Adsorption of the coliphages involves a primary reversible phase which requires the existence of complementary electrostatic and geometric relationships between host cell and virus (42 to 45). The first of these is realized by the presence, in the adsorbing medium, of cations of a valence and concentration range characteristic for each virus, plus organic factors such as *l*-tryptophan in certain instances. With T1 and T2, chemical studies indicate that it is the amino or substituted amino and carboxyl groups which are principally concerned in the initial binding. In T2, the positively charged amino groups of the virus particle appear to bind negative carboxyl radicals on the bacterial cell, while in T1, both types of groups on both surfaces apparently participate in the reaction. Sulfhydryl, phosphoric acid, and phenolic hydroxyl groups appear to play no major role in this primary binding.

This reversible binding of the phage is followed by an irreversible attachment. With T7 (46), one can distinguish two phases in this period: the first of these involves the irreversible binding of the virus, occurs at a rate dependent on the temperature of the environment, and is independent of metabolic activity on the part of the host cell since it can occur in heat-killed cells. The second phase involves fragmentation or splitting of the virus particle,

depends on the temperature of the medium, and in the case of T7, does not occur with heat-inactivated bacterial cells. With T2, however, Puck (45) finds that splitting occurs also with heat-killed cells, and he concludes that the release of DNA from the adsorbed phage is a direct effect of the establishment of the initial electrostatic bonds and does not require action of intracellular enzymes. Three alternative theories of the two-step nature of bacteriophage adsorption have been presented by Stent & Wollman (47). In the case of the lactic streptococcus bacteriophage, a specific requirement for calcium ions exists not only for adsorption but for maximal phage reproduction as well (47 to 50).

It is also possible to study the interaction between the coliphages and bacterial cells using so-called bacterial membranes. These are prepared by autolysis of heavy suspensions of bacteria in buffer solutions and subsequent treatment with proteolytic enzymes and with lysozyme. Such preparations are homogeneous and specifically adsorb and inactivate coliphages that attack the original bacterial cell (51, 52, 53). Membranes with comparable properties towards the phage can also be prepared from *Staphylococcus* K (54). In the case of the coliphages, Weidel has studied electron microscope pictures of the interaction between phage and membranes and finds that a dissolution of the latter occurs. Barrington & Kozloff, using membranes containing N^{15} (55) have more recently demonstrated that the interaction of specific phages with the insoluble membranes releases quantities of soluble nitrogenous compounds from the membranes in proportion to the quantity of phage added. While the relation of this phenomenon to *in vivo adsorption* is not clear, it is a matter of considerable interest since it is amenable to chemical investigation.

More detailed chemical information is available in regard to the interaction between several of the T coliphages and the dysentery bacillus, phase II *Shigella sonnei* (56, 57, 58). A specific lipocarbohydrate which is a component of the antigenic complex present on the surface of the bacterial cell can be prepared. When this is added to T4 in an appropriate medium, the contents of the viral membrane are promptly released, although the lipocarbohydrate itself is apparently not destroyed in the process.

Under normal physiological conditions, at 37°C. and in the presence of an adequate supply of nutrients, the splitting of the coliphage particle involves the transfer of the bulk of the nucleic acid of the phage into the bacterial cell, while the protein membrane remains attached to the exterior of the cell and can be removed by simply agitating the infected particles in the Waring Blendor without interfering with the further stages of virus reproduction (59). However, even prior to the clear demonstration of separate functions for phage DNA and protein, chemical evidence indicated that the phage particle was fragmented during infection. In experiments with P^{32}-labeled phage, Putnam & Kozloff (60) found phage fragments in bacterial lysates, and Lesley and co-workers (61) observed a splitting off of P^{32} during the pre-lysis period. Kozloff (62), using phage labeled with P^{32} and N^{15}, found

both labels appearing in the medium soon after infection and before lysis, indicating that both phage protein and DNA were broken down.

It is not possible at present to say that all of the phage DNA is transferred into the cell and that all of the phage protein is excluded. Whether the phage materials found in the medium before lysis result from the leaking out of DNA already injected into the bacterial cell, or whether the phenomenon has some other physiological significance is uncertain. So far as phage protein is concerned, certainly the bulk and possibly the whole is excluded from the cell. Using N^{15}-labeled T7 (46) (which does not form ghosts which might be reisolated and contaminate the viral progeny), parent nitrogen is found in the progeny, although the amount is small.

The release of phage phosphorus described above is to be distinguished from the so-called "stimulation breakdown" (61, 63) of secondarily infecting virus particles; i.e., if cells infected with T_2r^+ are superinfected with additional phage 3 min. after the initial infection, extensive breakdown of the superinfecting particles occurs with liberation of phosphorus into the medium and with the exclusion of this phosphorus from the viral progeny.

The further events involved in the formation of mature virus particles from the effective portion of the infecting particle can be followed by a number of procedures. It is possible to break open the bacterial cell by a variety of methods: sonic vibration (64, 65), lysis from without by using an excess of a different phage (64), mechanical disruption of the cells by decompression (66, 67, 68), or the addition of metabolic inhibitors such as cyanide (64) or proflavine (69). In all cases one finds that no phage particles can be recovered during the first half of the latent period, i.e., the time normally required for bacterial lysis. After this "eclipse" period, increasing amounts of active phage may be obtained. If one examines, by means of the electron microscope, the materials liberated by premature lysis, one finds that they contain a number of biologically inactive, tailless particles or "doughnuts," in numbers approximately equal to the active phage particles that would have been produced at normal lysis time (70). These doughnuts contain appreciable quantities of protein, and little or no nucleic acid. Although they are not adsorbed by bacteria, they fix complement with antiphage-antiserum but do not combine with phage-neutralizing antibody. Prematurely lysed cells also contain tailed particles which are devoid of nucleic acid. Counts of the doughnuts and of the tailed particles from bacteria disrupted at various times reveal an increase in doughnuts, followed by an increase in the tailed forms, suggesting the transformation of the doughnuts into the tailed particles. Experiments with S^{35} (71) indicate the presence in both premature and ordinary T4 lysates of noninfectious particles containing sulfur. These adsorb on bacteria and precipitate with anti-T4 serum in a way similar to phage. However, they are "somewhat lighter," are apparently devoid of nucleic acid, and have no appreciable killing effect on bacteria. In this latter respect, they differ from the ghosts formed by osmotic shock. In addition, a serologically specific ultrafilterable factor which will combine with phage neutralizing

antibody has been observed in lysates from the eclipse period (70). All of this strongly suggests that we are concerned with various intermediate stages in the synthesis of the final mature virus particle.

It is to be remarked that the facts listed above pertain to substances which are free of DNA. However, as will be discussed in more detail below, it appears that viral DNA appears within the infected cell within a few minutes after infection has occurred. In experiments involving P^{32}, Maaløe & Stent (72) found that the radioactivity which could be isolated by means of high speed centrifugation, antiphage serum precipitation, or specific adsorption to heat-killed bacteria from lysates in which intracellular phage development had been stopped at various stages with KCN, was associated with the appearance of plaque-forming particles. This suggests that phosphorus-containing phage structures do not take part in the formation of sedimentable, antigenically specific or adsorbing particles until very shortly before maturation. Phage formation then would involve the independent synthesis of various portions of the final particle with maturation involving a terminal putting-together of these component parts. However, one cannot exclude the possibility that the various protein-containing intermediate particles described above may contain DNA in the intracellular condition, but that the latter is lost during the process of premature liberation from the bacterial cell.

The important discovery of Wyatt & Cohen (73, 74, 75) that the even-numbered T phages contain a pyrimidine, 5-hydroxymethylcytosine, which is absent from the DNA of the bacterial host has offered a new method for studying the synthesis of viral DNA in infected bacteria. Measurement of phage specific DNA (i.e., of DNA containing 5-HMC[1]) and of host specific DNA (containing cytosine and no 5-HMC) at intervals after infection with T2 indicates that phage DNA begins to increase soon after infection while the bacterial DNA decreases (76). With the appearance of mature phage, phage DNA continues to increase and remains in excess of that present as infective particles. This excess DNA measures about 100 units per bacterium (the unit being defined as the DNA equivalent of one resting phage particle) from 25 min. after infection until the cell lyses. Moreover, the phosphorus of this surplus DNA is the phosphorus of the future virus particles, and it appears, therefore, that maturation (i.e., conversion of vegetative phage into the mature particle) is an irreversible process (77).

The mature virus particle may contain material derived from (*a*) the parent infecting particle, (*b*) the bacterial host cell, and (*c*) the nutrient components of the medium. So far as the contribution of the parental material is concerned, the following facts are paramount. By considering the relation between the transfer of parental material and burst size (78), or more directly by examination of phage obtained by premature lysis techniques (63, 79), it is clear that the parental contribution is confined to the virus particles formed early in the synthetic process. By the time 25 per cent of the virus yield is completed, most of the parental material has been transferred, and

the last virus particles synthesized contain little or no parental material. Further, the phosphorus transferred is not localized in specific or especially conserved parts of the DNA of the first progeny but is uniformly distributed in these particles (80). Experiments with parent virus labeled with both N^{15} and P^{32} show that the parental nucleic acid transferred to viral progeny contains relatively less of the original nitrogen than of the phosphorus (78). In the case of T7 (46), it can be calculated that the viral progeny contain about $2\frac{1}{2}$ atoms of parental nucleic acid nitrogen per atom of parental nucleic acid phosphorus. Since purine nucleotides contain 5 nitrogen atoms per phosphorus atom, and pyrimidine nucleotides contain on the average $2\frac{1}{4}$ nitrogen atoms per phosphorus atom, and in view of the relatively large direct contribution of parental purines to the progeny (79), it follows that a considerable fraction (possibly 30 or 40 per cent) of the parental phosphorus found in the progeny was transferred in some form other than as a nucleotide or polynucleotide. Moreover, it is also possible to demonstrate transfer of parental material in the absence of genetic transfer. Two types of experiments demonstrate this point. Various labeled bacteriophages (63, 78, 79), inactivated by ultraviolet light or x-rays, when mixed with live virus (unlabeled) and used to infect bacteria are found to transfer parental nucleic acid to the progeny in quantities that are somewhat smaller but still comparable to those transferred by nonirradiated virus particles. Further, a number of investigators (63, 78, 79) have demonstrated the transfer of considerable quantities of P^{32} between genetically unrelated viruses. For example, cells infected primarily with T7 and then infected secondarily with labeled T_6r^+ are found to produce T7 progeny containing significant quantities of the label. Up to the present no positive correlation has been obtained between material transfer from the infecting phage and the transfer of hereditary units. If special parts of the virus are conserved and transferred to the progeny as nucleic acid fragments of considerable size and possessing genetic specificity, no experiment has yet revealed their existence. The available data can be reconciled with the view that after the effective portion of the virus has exerted its effect on the metabolism of the host cell, it is broken down into smaller fragments which are then used for virus synthesis in a nonspecific manner.

So far as the contribution of the culture medium and the host are concerned, it is convenient to summarize our information in diagrammatic form (Figures 2 and 3) (81). The similarity of the data for T2, T4, and T6 permits use of a single figure to illustrate the origin of viral material in all three systems. After infection with these phages, most of the virus protein is synthesized *de novo* from the components of the medium. Experiments with N^{15} (82) and with lysine marked with C^{14} (83) demonstrate that about 6 to 12 per cent of the viral protein is derived from host protein; more particularly, the experiments with lysine show that this originates from host protein rather than from the amino acid pool. From the relationships between the concentration of host nitrogen in viral protein and burst size, it appears that most of the host contribution is to the first virus particles that are formed in the cell (82).

Apparently, when a limited source of host protein is used up, no further release of amino acids from the large amount of host protein occurs. It has also been shown that host cell protein nitrogen is not used for the synthesis of viral nucleic acid (84). From these facts, it appears that bacterial protein does not serve as a major source of material for viral synthesis.

A considerable amount of evidence indicates the conversion of host DNA into viral DNA in the T2, T4, and T6 systems (85). It was first shown by

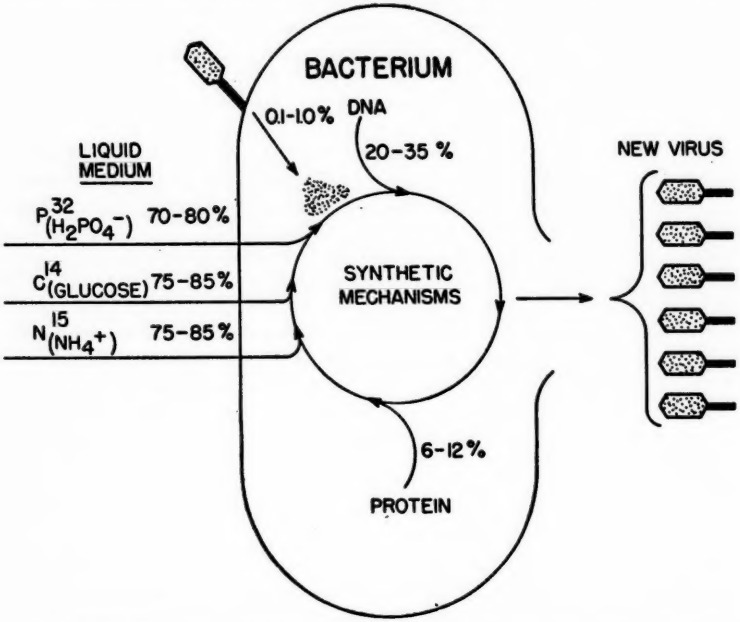


FIG. 2. Diagrammatic representation of the origin of the phosphorus, nitrogen and carbon of bacteriophages T2, T4, and T6. Percentages show origin of material found in viral offspring.

Cohen that host phosphorus was used for virus synthesis (86). The phosphorus was then identified as host DNA (83, 87). Isotopic tracer studies demonstrated the transfer of host purines (84) and pyrimidines (88) to viral DNA. In this connection, experiments using labeled host thymine (89) are definitive since thymine is present only in the DNA fraction of the host cell. The amount of host DNA transferred to progeny is large, as much as 100 per cent of the adenine of the bacterial DNA, 75 per cent of the guanine, and about 70 per cent of the host thymine. Experiments with labeled thymine also show that there is little or no conversion of thymine to 5-HMC in the

infected cell. Since a portion of the viral 5-HMC is derived from host pyrimidines (90), it appears that the source of this material must be host cytosine.

The molecular nature of the intermediates formed when host DNA is transferred to viral DNA is not known. The available evidence indicates that host DNA is degraded shortly after infection and that various DNA fragments are then used for the synthesis of viral DNA. It has been found, for

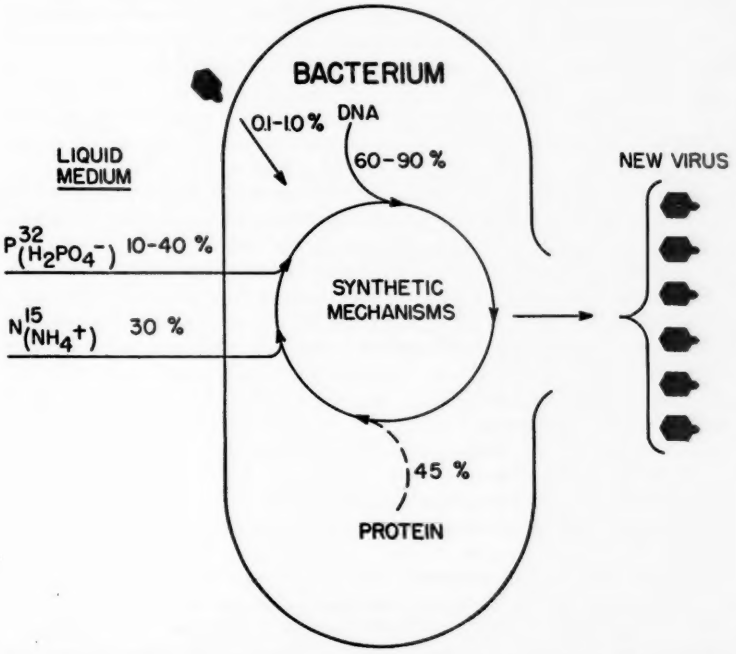


FIG. 3. Diagrammatic representation of the origin of the phosphorus and nitrogen of bacteriophage T7. Percentages show origin of material found in viral offspring.

instance, that 30 to 40 per cent more nitrogen than phosphorus of the host nucleic acid appears in the virus (82). In view of the fact that host DNA contains cytosine, while in T2, T4, and T6 cytosine is replaced by 5-HMC, it seems unlikely that relatively large fragments of host DNA could be used as such for virus synthesis. The transfer of both host bases and phosphorus suggests that some of the intermediates may be nucleotides or oligonucleotides, a view which is supported by the experiments excluding the free bases, adenine and guanine, as intermediates in the conversion of host DNA to viral DNA (84).

A diagrammatic representation of the origin of the nitrogen and phosphorus of the small short-tailed bacteriophage T7 is shown in Figure 3 (91, 92). Isotopic evidence indicates that 60 to 90 per cent of the viral DNA phosphorus comes from the host and the rest from the medium. There are three reasons for believing that host cell DNA is used for the synthesis of viral nucleic acid: (*a*) In synthetic medium there is little or no synthesis of DNA in a T7 infected cell; The host cell contains DNA equivalent to about 150 T7 particles and burst sizes of 100 to 120 are usually obtained; (*b*) Experiments with host cells differentially labeled in their various phosphorus components with P^{32} and infected in an isotope-free medium show that host cell DNA P^{32} is the major precursor of the P^{32} found in the virus; (*c*) The host cell contributes nitrogen as well as phosphorus to the nucleic acid of the viral progeny. Approximately equivalent amounts of viral nucleic acid nitrogen and phosphorus are furnished by the host cell. This suggests that the host cell contribution is largely in the form of nucleotides or polynucleotides. It is of some interest that the DNA of T7 contains the same four nitrogenous bases as the host DNA.

About one-half of the nitrogen of T7 protein is assimilated from the medium, while the remaining portion comes from the host cell. The actual source of the nitrogen contributed to T7 protein by the host cell has not been determined. By analogy to the T6 system, it appears that host protein probably is the source of the host nitrogen found in the virus protein.

The chemical and enzymic changes occurring in the infected bacterial cell have been only partially described. The chromatinic bodies of the bacterial host undergo a series of changes and the cells become enlarged and swollen (93, 94, 95). The oxygen uptake is unchanged, but Cohen has shown (96) that the metabolism of both the phospholipid and the RNA^1 fraction appears to cease inasmuch as these constituents of the host cell show no further incorporation of radioactive phosphate after infection. This is also true of the adenine and guanine of the RNA of the infected cell (84). Hershey has recently stated that he finds a metabolically active RNA fraction in T2 infected cells (77). Cohen (97) however, questions the latter's interpretation of his findings. In any case, the total DNA content increases as well as the protein. With T6 and the other tailed coliphages, there is insufficient bacterial DNA to supply that required by the virus, and a synthesis of considerable quantities of viral DNA from the components of the medium also takes place.

This involves, of course, an increased synthesis of desoxypentose, and Cohen has shown that this is effected by an alteration in the normal carbohydrate metabolism of the bacterial cell (97). In the normal growing cell, ribose is apparently formed by direct oxidation of glucose while desoxypentose is derived from the condensation of acetaldehyde with triosephosphate originating from glucose by way of the anerobic mechanisms of the Meyerhof-Embden scheme. The triose may also be formed by the oxidation of ribosephosphate. However, in the infected bacterial cell (with coliphage T6) the

oxidative path is sharply diminished and large quantities of hexosephosphate are converted into the necessary desoxypentose by way of the anerobic mechanism. It has already been pointed out that viral DNA in the T-even phages is characterized by the presence of 5-HMC. It has been suggested (97) that the shift in metabolic systems from cytosine to HMC synthesis accounts for the inability of the host to synthesize RNA. If this were true, virus infection in which host nucleic acids continue to be synthesized should not involve synthesis of HMC. In lysogenic systems, RNA is synthesized during virus reproduction and the temperate phages thus far examined do not contain 5-HMC (98). 5-Hydroxymethylcytosine also appears to be absent from the virulent phages T7 and T5, but Cohen believes that an inhibition of RNA synthesis in cells infected by these phages has not been demonstrated (97). In a continued study of the nature of the inhibition of RNA synthesis in infected cells, an examination has been made of the status of the enzymic mechanisms known to be involved. During infection, these mechanisms still are intact in the cell and are apparently uninhibited so that interference with RNA synthesis must involve other cellular factors (99). Manson, using a glycine-requiring mutant of *Escherichia coli* and studying the incorporation of marked glycine into purines, has concluded that RNA is "a metabolic dead-end," i.e., the rate of renewal of RNA in cells that are not growing is so slow that there is no need to postulate a specific inhibition of RNA synthesis during infection (100).

Changes in nucleic acid content can be demonstrated in the absence of actual viral reproduction. Herriott and his collaborators (101) have found that infection with the DNA free "ghosts" formed by osmotic shock treatment of T2 also causes a block in RNA synthesis although both DNA and protein are synthesized in appreciable quantities. Whether the DNA formed under these circumstances is characteristic of the virus or of the host has not yet been ascertained.

Changes in the enzymic spectrum of bacterial cells infected with phage have been noted by various investigators (40, 102). Both Pardee, who was studying a series of enzymes (103, 104), and Kozloff have found that desoxyribonuclease activity increases two- to threefold in the infected cell. This increase in activity can be ascribed primarily to a decrease in the presence of a naturally occurring inhibitor (81). In the uninfected cell, the inhibitor is present in excess, and little or no desoxyribonuclease activity can be demonstrated in normal cells. As infection proceeds, the concentration of this inhibitor decreases, although some still remains at the moment of lysis. The inhibitor is heat stable, nondialyzable, unaffected by shaking with chloroform, and is apparently a specific ribose nucleic acid component of the bacterial cell, which can be inactivated by ribonuclease. Centrifugal fractionation of sonic extracts of *E. coli* shows that the inhibitory RNA comprises a small fraction of the total RNA of the cell. The action of the inhibitor is specific, inasmuch as desoxyribonuclease from pancreas is unaffected by it.

Temperate phages.—The subject of lysogeny has been recently and thor-

oughly reviewed by Lwoff (105), and only certain aspects of the phenomenon can be discussed here. When a bacterial cell is exposed to a temperate phage a variety of interactions may take place. The bacteria may be nonresponsive and remain entirely uninfected. In other cases, infection may occur but the phage disappears in an unknown fashion, i.e., the infection has been aborted. An aborted infection also may occur with killing of the bacterium and no production of phage. Under other circumstances, phage is reproduced with lysis of the bacterium, i.e., the phage behaves as a virulent virus and the situation is comparable to that described in the previous section. In the final circumstance the temperate phage is adsorbed and the bacterium survives to give rise to a lysogenic clone. Here the phage particle is "reduced" to a prophage. The host cell continues to grow and reproduce in a normal fashion, with the prophage also being reproduced as an essential component of each daughter cell originating from the parent. In growing cultures of such cells one finds that in a small proportion of the cells the prophage is converted spontaneously into an active vegetative particle which proceeds then to develop into mature phage. This results in the lysis of the particular cells concerned, with the liberation of the phage particles into the medium. With certain prophages and certain lysogenic strains, it is possible to cause a practically complete conversion of the prophage by treating the lysogenic cells with one of a variety of so-called inducing agents. When such substances are applied under the proper conditions, conversion of the prophage to the vegetative state occurs followed by practically complete lysis of the cultures and liberation of large amounts of virus particles. The phage produced under these circumstances is identical with the original infecting particle producing the prophage, and when adsorbed in the same host will undergo the same series of changes.[2]

As one might expect, this general pattern can be modified by changes in the variables involved in each phase of the process. Even if the phage is of the necessary genetic strain, it is possible, by changing the temperature at which the cells are maintained (108) or by altering the multiplicity of the infection (106), to cause the virus to behave in a virulent rather than temperate fashion. Under circumstances in which reduction to the prophage state occurs, it is possible to modify this. With *E. coli* K12 S strain (109) exposed to the temperate phage lambda, reduction requires something less than 1 hr. at 37°C. During this period the intracellular phage is more easily heat-inactivated than free phage, so that it is possible during the reduction period to rid cells of their potential prophage by subjecting them to higher temperatures. On the other hand, once established, the prophage is even more resistant to heat-inactivation at 43°C. than is free phage. Even when lysogenization has been accomplished, this can in some cases be reversed by changing

[2] The general picture of lysogeny given here is largely that of Lwoff and his collaborators and has been questioned by Boyd (106). Also, Parry & Edwards (107)have reported data with *S. typhimurium* that they believe cannot be reconciled with either view.

the media in which the material is cultured (110, 111), e.g., if lysogenic *B. megaterium* is cultured in a synthetic medium containing citrate (112), lysogenicity is lost after 61 subcultures.

The nature of the conversion of phage to prophage, i.e., the reduction process, is not clear but it appears that during the period when reduction is occurring the phage particle behaves as a cytoplasmic unit, while after the prophage status has been established, it is apparently firmly associated with or bound to a specific chromosomal site (109).

After infection with a temperate phage, one observes a delay in the division of the infected cell (113) and transient morphological changes may also occur (114). Eventually the cells become cytologically normal again and divide regularly.

At present we have no precise information as to the chemical nature of the prophage. Lanni has looked for phage antigens in sonic vibrated preparations of *S. dysenteriae* (P2) (115). No antigen was discerned under conditions such that a serological yield equivalent to about one phage particle per 20 bacteria could reasonably have been measured. It thus appears that the lysogenic bacteria (P2) do not contain the serological equivalent of the serum-blocking power of the tail protein of the mature phage P2. It appears therefore that this specific protein is synthesized only when the lysogenic bacteria are induced to form bacteriophage. Since nucleic acids are devoid of antigenic properties, it is attractive to postulate that the prophage might be a nucleic acid, comparable perhaps to the material injected into the bacterial cell by the virulent phages.

A considerable body of genetic evidence supports the view that the prophage is a gene-like unit located at a specific chromosomal site. Experiments designed to measure the number of prophages present in bacterial cells (108, 116), observations on the transmission of lysogeny during bacterial crosses (117, 118, 119), experiments in which induced lysogenic bacteria are infected with virulent phage (120), experiments involving lysogenic bacteria carrying two related prophages (121), and, according to Lwoff (105), the available data concerning lysogenization, incompatibility, and immunity, can all be interpreted from this point of view.

With many prophage-containing cells, it is possible under the correct environmental conditions to cause a conversion of prophage into the vegetative state. However, not all prophage-carrying bacterial strains are capable of induction, nor do all inducible strains respond to the same agent. Of the latter, ultraviolet light with a wave length of around 2537 (122) and soft x-rays are very efficient (123), also γ rays of radioactive cobalt (105), thiomalic acid (124), reduced glutathione (124), ascorbic acid (124), organic peroxides (125), epoxides (125), ethyleneimines (125), nitrogen mustards (126, 127), and hydrogen peroxide (124), either added directly or produced by the addition of sulfhydryl compounds in the presence of copper. The mode of action of these agents is not known, and it is uncertain whether all of them behave in the same way. Lwoff is of the opinion that their effect is primarily

to cause a disturbance in the nucleic acid metabolism of the lysogenic bacteria rather than to alter directly the prophage itself (105).

If induction occurs, bacterial growth proceeds without bacterial division during a latent period corresponding to one or two generations. No phage is released during this period, but if the induced lysogenic bacteria are prematurely disrupted by lysozyme or cyanide, new mature virus particles appear at the end of the second third of the latent period, and increase linearly until a full yield is reached (122). Many features of phage multiplication in induced lysogenic bacteria coincide almost exactly with those observed after infection of nonlysogenic bacteria with a virulent phage. Thus, Jacob & Wollman (116) have compared the multiplication of *Pseudomonas aeruginosa* (*pyocyanea*) phage P8 after infection of sensitive bacteria 13 with the synthesis of the same phage after ultraviolet induction in lysogenic bacterial strains. Such characteristics of phage development as average burst size, distribution of individual burst sizes, etc., are identical in both systems with the exception of the length of the minimum latent period, which is longer in induced lysogenic than in infected sensitive bacteria, even when these sensitive bacteria have been previously irradiated with the same dose of ultraviolet light as is used for induction of lysogenic bacteria.

In spite of superficial resemblance between the two systems, there are marked differences in the chemical and enzymic composition of the induced bacterial cell as compared with the bacterium infected with virulent phage. After a prophage-carrying bacterial cell has been induced, the oxygen uptake of the cell continues to increase and the cell continues to grow although cell division does not occur (122). Unlike T-even infected *E. coli*, the induced cells continue to synthesize RNA (128) and are capable of forming adaptive enzymes almost up to the time mature virus particles can be detected in the cell (129, 130). One also observes the increased synthesis of DNA and protein which is a common feature of both types of viral infection, although there is a delay in the synthesis of DNA during the first phase of the latent period in the induced cells.

It is not clear why the virulent phages cause a more extensive inhibition of the synthetic reactions of the infected cell. Jacob has studied this phenomenon, comparing the infection of *P. aeruginosa* with the virulent phage P2 to infection with the temperate phage P8 (131). In his experiments, glucose served as the sole carbon source for the bacteria and could be introduced at a controlled rate. When the available glucose concentration was reduced beyond a certain point, bacterial growth was retarded. However, in bacteria infected by the virulent phage, it was possible to reduce the amount of available glucose to one-third or one-fourth of that necessary for growth without affecting the yield of virus per bacterial cell. It was only after the environment contained less than one-fifth of the carbon necessary for bacterial growth that the yield of phage particles per cell declined.

In *P. aeruginosa* infected with the temperate phage P8 and induced with ultraviolet light, phage synthesis, as with virulent phage, is the last process

to be affected by a decrease in the concentration of available glucose. As the glucose concentration of the medium is lowered, RNA synthesis and the ability of the cell to form adaptive enzymes are all decreased. However, the phage yield remained unchanged, and it was necessary to lower the concentration of glucose available to one-third to one-fourth of that required for growth before the synthesis of virus was decreased. Two conclusions derive from those experiments: first, that in spite of the synthetic limitations that occur with the virulent phages, apparently a considerable portion of the metabolic activity of the infected cell is not directed to virus synthesis, and second, that in the case of both virulent and temperate phages, phage synthesis has a preferential claim on the energy and material sources available to the infected cell.

Aptitude or the ability to be induced by ultraviolet light or other inducing agents depends, among other factors, on the nutritional status of the organism at the time of treatment. With *B. megaterium* 899, induction occurs only if the bacteria are grown in yeast extract and not in a synthetic medium (122). With *P. aeruginosa*, glucose starvation causes a loss in aptitude(132). A similar effect of starvation is observed in *E. coli* infected with λ (133). In the same fashion, phage synthesis can be shown to have specific requirements in respect to the organic (134) or inorganic (135) constituents of the medium. Optimal yields of λ phage from K12 can be obtained only when leucine and valine are present (136). Isoleucine is also essential, but it can be shown that it is required to prevent an inhibitory effect of valine. This is also true for a threonine requirement which is associated with an inhibitory action exerted by cysteine, although the parent lysogenic K12 strain requires none of these amino acids for normal growth and can multiply on a medium containing inorganic nitrogen. This is the antithesis to the situation described for a thymine-requiring mutant of *E. coli*. When this strain was multiply infected with virulent T2, virus was formed even though thymine was excluded from the medium (97).

General Remarks

In reviewing the literature of the last few years, emphasis has been placed on those facts which seem most pertinent to a biochemical understanding of virus synthesis. A number of topics have not been discussed: the effects of antimetabolites and antibiotics on phage growth (137, 138); the effects of radiation on phage or infected bacteria (139 to 142); and the considerable literature on the genetic aspects of bacteriophage growth. While the geneticist is able to describe the process of virus reproduction in specific terms, these cannot be translated at the moment into comparable chemical structures. Visconti & Delbruck (143), studying genetic recombination in phage, postulate an initial conversion of the parental particle to a vegetative state. The vegetative particles multiply and mate pair-wise repeatedly and, with respect to partner, at random. Maturation of the vegetative particles into the mature particles is irreversible and the mature particles do not multiply

nor mate. Visconti & Delbruck have carried out experiments for 2- and 3-factor crosses, and find the results agree with the theory if the average number of matings was taken to be five. Levinthal & Visconti (144) have reported data that are consistent with such a hypothesis if it is assumed that the pool of mating vegetative noninfectious phage particles remains constant at a size of about 30 particles. These figures are not too remote from those obtained by Hershey for the number of vegetative particles present in the infected virus cell. However, there are many aspects of the biological behavior of the phages that would seem unlikely to offer much hope for direct chemical attack at the moment, e.g., the transitory host induced mutation described by Luria and by others (145 to 148).

It becomes clear that one may no longer regard the bacterial viruses simply as lethal agents, reproducing themselves at the expense of a parasitized host. Rather, the host cell-virus complex constitutes a new biological entity, and as Luria has said (12) one may

> visualize a series of alternative rulers of cell destiny—the bacterial nucleus, the prophage, and the exogenous phage. The prophage of a temperate phage can displace the bacterial pattern without help from inducing agents, and in induced bacteria the latent prophage captures the lead, not immediately upon induction but after an interval during which it can still be displaced by an exogenous phage.

The association between the bacterial viruses and the genetic material of the cell, suggested by chemical similarities, becomes more defined as the biological behavior of these particles is further explored. Infection with bacteriophage can be demonstrated to involve the transformation of nontoxic strains of diphtheria bacteria into toxic strains (148, 149, 150). It is clear also that phage particles are associated with the transduction of genetic characteristics (151 to 154) and with the F factor which is responsible for polarity in genetic recombination in *E. coli* (155, 156). Just as the bacterial viruses represent a group of biologically active nucleic acid-protein complexes secreted or manufactured by the cell itself, we recognize also the existence of other biologically active particles formed by the bacterial cell which are either DNA or protein. The transforming factors which are capable of altering the genetic material of the infected bacterial cell appear to be entirely DNA (157). On the other hand, the colicines (158) are DNA-free proteins capable of killing bacterial cells but unable to reproduce themselves, although their formation can be induced in bacterial strains by the action of ultraviolet light. It seems certain that the biology of the nucleoproteins involves a whole spectrum of phenomena, of which as yet we have recognized only a portion.

LITERATURE CITED

1. Price, W. H., *Ann. Rev. Microbiol.*, **6**, 333 (1952)
2. *The Nature of Virus Multiplication* (Fildes, Paul, and Van Heyningen, W. E., Eds., Cambridge University Press, Cambridge, England, 320 pp., 1953)

3. *Le Bacteriophage—Colloque de Royaumont* (Institut Pasteur, Secretariat General de l'Union Internationale des Sciences Biologiques, Paris, France, 318 pp., 1953)
4. *Viruses: Cold Spring Harbor Symposia Quant. Biol.*, **18**, (1953)
5. *The Dynamics of Virus Infection* (Presented at International Symposium, Detroit, Mich., October, 1953; Hartman, Frank W., Horsfall, Frank L., Jr., and Kidd, John G., Eds., Blakiston Co., Inc., Philadelphia, Penna., 1954)
6. Kozloff, L. M., *Exptl. Cell Research*, Suppl. 2, 367 (1952)
7. Putnam, F. W., *Exptl. Cell. Research*, Suppl. 2, 245 (1952)
8. Graham, A. F., *Can. J. Med. Sci.*, **31**, 279 (1953)
9. Putnam, F. W., *Advances in Protein Chem.*, **8**, 177 (1953)
10. Epstein, H. T., *Advances in Virus Research*, **1**, 1 (1953)
11. Evans, E. A., Jr., *Biochemical Studies of Bacterial Viruses* (University of Chicago Press, Chicago, Ill., 68 pp., 1952)
12. Luria, S. E., *General Virology* (John Wiley & Sons, Inc., New York, N. Y., 427 pp., 1953)
13. Anderson, T. F., *Am. Naturalist*, **86**, 91 (1952)
14. Noda, H., and Wyckoff, R. W. G., *Biochim. et Biophys. Acta*, **8**, 381 (1952)
15. Noda, H., *Biochim. et Biophys. Acta*, **12**, 495 (1953)
16. Beutner, E. H., Hartman, P. E., Mudd, S., and Hillier, J., *Biochim. et Biophys. Acta*, **10**, 143 (1953)
17. Mudd, S., Hillier, J., Beutner, E. H., and Hartman, P. E., *Biochim. et Biophys. Acta*, **10**, 153 (1953)
18. Fraser, D., and Williams, R. C., *J. Bact.*, **65**, 167 (1953)
19. Williams, R. C., and Fraser, D., *J. Bact.*, **66**, 458 (1953)
20. Williams, R. C., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 185 (1953)
21. Anderson, T. F., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 197 (1953)
22. Anderson, T. F., *Ann. inst. Pasteur*, **84**, 5 (1953)
23. Anderson, T. F., *Botan. Rev.*, **15**, 464 (1949)
24. Fraser, D., and Jerrel, E. A., *J. Biol. Chem.*, **205**, 291 (1953)
25. Herriott, R. M., and Barlow, J. L., *J. Gen. Physiol.*, **36**, 17 (1953)
26. Fraser, D., and Williams, R. C., *Proc. Natl. Acad. Sci. U. S.*, **39**, 750 (1953)
27. Lanni, F., and Lanni, Y. T., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 159 (1953)
28. Friedman, M., and Cowles, P. B., *J. Bact.*, **66**, 379 (1953)
29. Friedman, M., *J. Bact.*, **66**, 501 (1953)
30. Anderson, E. S., and Felix, A., *J. Gen. Physiol.*, **9**, 65 (1953)
31. Baer, B. S., and Krueger, A. K., *J. Gen. Physiol.*, **35**, 857 (1953)
32. Baer, B. S., and Krueger, A. K., *J. Gen. Physiol.*, **36**, 111 (1953)
33. Baer, B. S., and Krueger, A. K., *J. Gen. Physiol.*, **36**, 127 (1953)
34. Beumer, J., and Beumer-Jochmans, M. P., *Ann. inst. Pasteur*, **83**, 582 (1952)
35. Beumer, J., and Beumer-Jochmans, M. P., *Ann. inst. Pasteur*, **84**, 328 (1953)
36. Giantini, J., Edlinger, E., and Nicolle, P., *Ann. inst. Pasteur*, **84**, 787 (1953)
37. Edlinger, E., and Giantini, J., *Ann. inst. Pasteur*, **84**, 969 (1953)
38. Farrant, J. L., and Rountree, P. M., *J. Gen. Microbiol.*, **9**, 288 (1953)
39. Elford, W. J., Guelin, A. M., Hotchin, J. E., and Challice, C. E., *Ann. inst. Pasteur*, **84**, 319 (1953)
40. Joklik, W. K., *Brit. J. Exptl. Pathol.*, **33**, 368 (1952)
41. Adams, M. H., *J. Bact.*, **64**, 387 (1952)

42. Garen, A., and Puck, T. T., *J. Exptl. Med.*, **94**, 177 (1951)
43. Puck, T. T., Garen, A., and Cline, J., *J. Exptl. Med.*, **93**, 65 (1953)
44. Puck, T. T., and Sagik, B., *J. Exptl. Med.*, **98**, 808 (1953)
45. Puck, T. T., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 149 (1953)
46. Mackal, R. P., and Kozloff, L. M., *J. Biol. Chem.* (In press)
47. Stent, G. S., and Wollman, E. L., *Biochim. et Biophys. Acta*, **8**, 250 (1952)
48. Potter, N. N., and Nelson, F. E., *J. Bact.*, **64**, 105 (1952)
49. Potter, N. N., and Nelson, F. E., *J. Bact.*, **64**, 113 (1952)
50. Potter, N. N., and Nelson, R. E., *J. Bact.*, **66**, 508 (1953)
51. Weidel, W., *Z. Naturforsch.*, **6b**, 251 (1951)
52. Weidel, W., *Ann. inst. Pasteur*, **84**, 60 (1953)
53. Weidel, W., *Cold Spring Harbor Symposia Quant. Biol.*, **18** 155 (1953)
54. Hotchin, J. E., Dawson, I. M., and Elford, W. J., *Brit. J. Exptl. Pathol.*, **33**, 177 (1952)
55. Barrington, L. F., and Kozloff, L. M., *Science* (In press)
56. Jesaitis, M. A., and Goebel, W. F., *J. Exptl. Med.*, **96**, 425 (1952)
57. Goebel, W. F., and Jesaitis, M. A., *Ann. inst. Pasteur*, **84**, 66 (1952)
58. Jesaitis, M. A., and Goebel, W. F., *Cold Spring Harbor Symposia Quant. Biol.* **18**, 205 (1953)
59. Hershey, A. D., and Chase, M., *J. Gen. Physiol.*, **36**, 39 (1952)
60. Putnam, F. W., and Kozloff, L. M., *J. Biol. Chem.*, **182**, 243 (1950)
61. Lesley, S. M., French, R. C., Graham, A. F., and van Rooyen, C. E., *Can. J. Med. Sci.*, **29**, 128 (1951)
62. Kozloff, L. M., *J. Biol. Chem.*, **194**, 83 (1952)
63. French, R. C., Graham, A. F., Lesley, S. M., van Rooyen, C. E., *J. Bact.*, **64**, 597 (1952)
64. Doermann, A. H., *J. Gen. Physiol.*, **35**, 645 (1952)
65. Anderson, T. F., and Doermann, A. H., *J. Gen. Physiol.*, **35**, 657 (1952)
66. Fraser, D., *Nature*, **167**, 33 (1951)
67. Levinthal, C., and Fisher, H. W., *Biochim. et Biophys. Acta*, **9**, 419 (1952)
68. Levinthal, C., and Fisher, H. W., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 29 (1953)
69. Foster, R. A. C., *J. Bact.*, **56**, 795 (1948)
70. DeMars, R. I., Luria, S. E., Fisher, H. W., and Levinthal, C., *Ann. inst. Pasteur*, **84**, 113 (1953)
71. Maaløe, O., and Symonds, N., *J. Bact.*, **65**, 177 (1953)
72. Maaløe, O., and Stent, G. S., *Acta Pathol. Microbiol. Skand.*, **30**, 149 (1952)
73. Wyatt, G. R., and Cohen, S. S., *Nature*, **170**, 1072 (1952)
74. Wyatt, G. R., and Cohen, S. S., *Ann. inst. Pasteur*, **84**, 143 (1953)
75. Wyatt, G. R., and Cohen, S. S., *Biochem. J.* (*London*), **55**, 774 (1953)
76. Hershey, A. D., Dixon, J., and Chase, M., *J. Gen. Physiol.*, **36**, 777 (1953)
77. Hershey, A. D., *J. Gen. Physiol.*, **37**, 1 (1953)
78. Kozloff, L. M., *J. Biol. Chem.*, **194**, 95 (1952)
79. Watson, J. D., and Maaløe, O., *Biochim. et Biophys. Acta*, **10**, 432 (1953)
80. Maaløe, O., and Watson, J. D., *Proc. Natl. Acad. Sci. U. S.*, **37**, 507 (1951)
81. Kozloff, L. M., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 209 (1953)
82. Kozloff, L. M., Knowlton, K., Putnam, F. W., and Evans, E. A., Jr., *J. Biol. Chem.*, **188**, 101 (1951)
83. Siddiqi, M. S. H., Kozloff, L. M., Putnam, F. W., and Evans, E. A., Jr., *J. Biol. Chem.*, **199**, 165 (1952)

84. Koch, A. L., Putnam, F. W., and Evans, E. A., Jr., *J. Biol. Chem.*, **197**, 113 (1952)
85. Evans, E. A., Jr., *Ann. inst. Pasteur*, **84**, 129 (1953)
86. Cohen, S. S., *J. Biol. Chem.*, **174**, 295 (1948)
87. Kozloff, L. M., and Putnam, F. W., *J. Biol. Chem.*, **182**, 241 (1950)
88. Weed, L., and Cohen, S. S., *J. Biol. Chem.*, **192**, 693 (1951)
89. Banks, M., reported in *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 213 (1953)
90. Weed, L. L., and Courtenay, T. A., *Federation Proc.*, **12**, 465 (1953)
91. Putnam, F. W., Miller, D., Palm, L., and Evans, E. A., Jr., *J. Biol. Chem.*, **199**, 177 (1952)
92. Labaw, L. W., *J. Bact.*, **62**, 169 (1951)
93. Luria, S. E., and Human, M. L., *J. Bact.*, **59**, 551 (1950)
94. Murray, R. G. E., Gillen, D. H., and Hengy, F. C., *J. Bact.*, **59**, 603 (1950)
95. Murray, R. G. E., and Whitfield, J. F., *J. Bact.*, **65**, 75 (1953)
96. Cohen, S. S., *J. Biol. Chem.*, **174**, 381 (1948)
97. Cohen, S. S., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 221 (1953)
98. Smith, J. D., and Siminovitch, L. (unpublished) in *Bact. Rev.*, **17**, 320 (1953)
99. Cohen, S. S., and Roth, L., *J. Bact.*, **65**, 490 (1953)
100. Manson, L. A., *J. Bact.*, **66**, 702 (1953)
101. Herriott, R. M. (Unpublished data. Presented at Meeting of Soc. Amer. Bacteriologists, San Francisco, Calif., August, 1953)
102. Friedman, S., and Gots, J. J., *J. Biol. Chem.*, **201**, 125 (1953)
103. Pardee, A. B., and Kunker, R. C., *J. Biol. Chem.*, **199**, 9 (1952)
104. Pardee, A. B., and Williams, I., *Arch. Biochem. and Biophys.*, **40**, 222 (1952)
105. Lwoff, A., *Bact. Revs.*, **17**, 269 (1953)
106. Boyd, J. S. K., *J. Pathol. Bact.*, **63**, 445 (1951)
107. Parry, W. R., and Edwards, J., *J. Gen. Microbiol.*, **9**, 342 (1953)
108. Gertani, G., *Ann. inst. Pasteur*, **84**, 273 (1953)
109. Lieb, M., *J. Bact.*, **65**, 642 (1953)
110. dooren de Jong, L. E. den., *Zentr. Bakt. Parasitenik., Abt. I Orig.*, **120**, 1 (1950)
111. DeCarlo, M. R., Sarles, W. B., and Knight, S. G., *J. Bact.*, **65**, 53 (1953)
112. Clarke, N. A., *J. Bact.*, **63**, 187 (1952)
113. Bertani, G., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 65 (1953)
114. Murray, R. G. E., *Rept. Proc. 6th Intern. Congr. Microbiol.* (Rome, Italy, 1953) (In press)
115. Lanni, F., in Lwoff, A., *Bact. Revs.*, **17**, 269 (1953)
116. Jacob, F., and Wollman, E., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 101 (1953)
117. Lederberg, E. M., *Genetics*, **36**, 560 (1951)
118. Lederberg, E. M., and Lederberg, J., *Genetics*, **38**, 51 (1953)
119. Wollman, E., *Ann. inst. Pasteur*, **84**, 281 (1953)
120. Weigle, J. J., and Delbrück, M., *J. Bact.*, **62**, 301 (1951)
121. Appleyard, R. K., *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 95 (1953)
122. Lwoff, A. Siminovitch, L., and Kjeldgaard, N., *Ann. inst. Pasteur*, **79**, 815 (1950)
123. Latarjet, R., *Ann. inst. Pasteur*, **81**, 389 (1951)
124. Lwoff, A., and Siminovitch, L., *Ann. inst. Pasteur*, **82**, 676 (1952)
125. Lwoff, A., and Jacob, F., *Compt. rend.*, **234**, 2308 (1952)
126. Jacob, F., *Ann. inst. Pasteur*, **83**, 671 (1952)

127. Williams-Smith, H., *J. Gen. Microbiol.*, **8,** 116 (1953)
128. Siminovitch, L., and Rapkine, S., *Biochim. et Biophys. Acta*, **9,** 478 (1952)
129. Jacob, F., *Compt. rend.*, **232,** 1780 (1951)
130. Siminovitch, L., and Jacob, F., *Ann. inst. Pasteur*, **83,** 745 (1952)
131. Jacob, F., *Ann. inst. Pasteur*, **82,** 578 (1952)
132. Jacob, F., *Ann. inst. Pasteur*, **82,** 433 (1952)
133. Borek, E., *Biochim. et Biophys. Acta*, **8,** 211 (1952)
134. Lwoff, A., *Compt. rend.*, **234,** 366 (1952)
135. Huybers, K., *Ann. inst. Pasteur*, **84,** 242 (1953)
136. Gots, J. J., and Hunt, G. R., Jr., *J. Bact.*, **66,** 353 (1953)
137. Wooley, J. G., Murphy, M. K., Bond, H. W., and Perrins, T. D., *J. Immunol.*, **68,** 523 (1952)
138. Altenbern, R. A., *J. Bact.*, **65,** 288 (1953)
139. Watson, J. D., *J. Bact.*, **63,** 473 (1952)
140. Buzzell, A., and Lauffer, M. A., *Arch. Biochem. and Biophys.*, **39,** 195 (1952)
141. Labaw, L. W., Mosley, V. M., and Wyckoff, R. W. G., *J. Bact.*, **65,** 330 (1953)
142. Yanofsky, C., *J. Bact.*, **65,** 383 (1953)
143. Visconti, N., and Delbruck, M., *Genetics*, **38,** 1 (1953)
144. Levinthal, C., and Visconti, N., *Genetics*, **38,** 500 (1953)
145. Luria, S. E., and Human, M. L., *J. Bact.*, **64,** 557 (1952)
146. Bertani, G., and Weigle, J. J., *J. Bact.*, **65,** 113 (1953)
147. Fernandez, B., Haas, F. L., and Wyss, O., *Proc. Natl. Acad. Sci. U. S.*, **39,** 1052 (1953)
148. Freeman, V. J., and Morse, I., *J. Bact.*, **63,** 407 (1952)
149. Hewitt, L. K., *Lancet*, **II,** 272 (1952)
150. Groman, N. B., *J. Bact.*, **66,** 184 (1953)
151. Zinder, N. D., and Lederberg, J., *J. Bact.*, **64,** 679 (1952)
152. Berry, M. E., McCarthy, A. M., and Plough, H. P., *Proc. Natl. Acad. Sci. U.S.*, **38,** 797 (1952)
153. Stocker, B. A. D., Zinder, N. D., and Lederberg, J., *J. Gen. Microbiol.*, **9,** 410 (1953)
154. Baron, L. S., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 271 (1953)
155. Hayes, W. J., *J. Gen. Microbiol.*, **8,** 72 (1953)
156. Hayes, W. J., *Cold Spring Harbor Symposia Quant. Biol.*, **18,** 75 (1953)
157. Austrian, R., *Bact. Revs.*, **16,** 31 (1952)
158. Fredericq, P., *Ann. inst. Pasteur*, **84,** 294 (1953)

CHANGES IN THE HOST FLORA INDUCED BY CHEMOTHERAPEUTIC AGENTS[1]

By Elizabeth McCoy

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin

There is no universal chemotherapeutic agent; nor is there any so specific as to attack only the pathogen in the complex microflora of the host. At best the chemotherapeutic agent is selective, chosen for its ability to suppress or kill the pathogen; whether it also kills certain other members of the host's flora is incidental. Actually it is often advantageous that the chemotherapeutic agent be able to kill a group of organisms, since it may thus be useful for treatment of various, even undiagnosed infectious processes. In the antibiotic field the term microbial spectrum has come to be used for that group of microorganisms upon which a given antibiotic is effective, i.e., inhibitory or lethal. For penicillin and erythromycin the spectrum includes principally gram-positive bacteria; for streptomycin both gram-positive and gram-negative bacteria (species of *Proteus*, *Pseudomonas*, and certain other genera excepted). Some antibiotics are known for their very "broad spectra," for example, chlortetracycline (aureomycin), oxytetracycline (terramycin) and chloramphenicol (chloromycetin). More limited spectra are also known, and in certain cases an antibiotic may have particular effectiveness which makes it the chemotherapeutic agent of choice for an otherwise stubborn infection; for example, polymyxin B for *Pseudomonas* spp. (1, 2), chloramphenicol for *Proteus* spp. (1), or neomycin alone (3) or with phthalylsulfathiazole (4) broadly for the coliform and other gram-negative organisms of the preoperative intestinal tract of man. Thus there exists a mosaic of specificities to characterize the various antibiotics. To the extent that the flora, after a course of antibiotic therapy, lacks the original susceptible forms, it differs from the host's original flora in respect to both the so-called normal nonpathogens and the pathogen in question. Secondary growth of the remaining flora may still further shift the population and create the so-called induced changes. Among the interesting and important changes are those involving (*a*) antibiotic resistant forms, (*b*) the rampant growth of previously minor members of the flora, and (*c*) the special case of the changed intestinal flora as it may affect vitamin synthesis and growth of the host. It is proposed to limit this review to these three aspects, around each of which there has accumulated a very extensive literature in the decade during which antibiotics have been widely used. For purposes of this review, however, citations will be taken mainly from 1953. This course seems justified because the evidence on changes in host microflora is cumulative, particularly on the subjects of emergence of resistant flora and the overgrowth of secondary flora following the overuse of antibiotics. Further, the literature

[1] The survey of literature pertaining to this review was completed in January, 1954.

of 1953 offers the best explanations so far advanced for the curious phenomenon of enhanced growth of certain young animals fed with antibiotics; there are also two excellent reviews (5, 6) on the subject. Chemotherapeutic agents such as sulfonamides, isoniazid, and *p*-aminosalicylic acid used alone or with the antibiotics will be mentioned where pertinent, but no attempt will be made to cover the entire subject of such chemotherapeutics, as they may exert selective action on the infectious process in the host.

Emergence of Resistant Microflora, in the Primary Host or in the General Population

It was inevitable that the phenomenon of resistance to antibiotics should create a therapeutic problem of increasing concern. This situation was recognized early, and it has been one factor in the emphasis upon discovery and use of new antibiotics. There are even occasional efforts to curb the use of an effective antibiotic to minimal effective dosage (7, 8), or even to ask the Council of Pharmacy and Chemistry of the American Medical Association to restrict use of some antibiotic, for example, neomycin to intestinal antisepsis at surgery (4). Something of the same reasoning is in the minds of the British (9, 10, 11) in questioning the feeding use of antibiotics in Great Britain. Porter (11) in particular urged that such use of antibiotics might result in increased resistance which would complicate veterinary practices. He recommended restriction to fattening pigs, poultry, and young calves, which are animals sent to slaughter rather than retained on the farm.

The potential seriousness of the resistant population is illustrated by reports from several large hospitals. Needham & Co-workers have reported several times upon the situation at Mayo Clinic hospitals. The 1953 report (12) containing general statements on incidence of *Micrococcus pyogenes* is shown in Table I. Similar values are given by Miyahara, Cariker & Clapper

TABLE I

Incidence of Resistant Strains of Micrococcus Pyogenes in Mayo Clinic Hospitals (12)

Resistant to:	1948	1951	1953
Penicillin	60%		
Streptomycin	41%		
Chlortetracycline (Aureomycin)	0%	36%	45%
Oxytetracycline (Terramycin)		36%	45%
Chloramphenicol (Chloromycetin)			1+%
Erythromycin			0%*

* Applies to status on admission to hospital: one case developed in hospital.

(13) for the Colorado General Hospital, Denver. Of the coagulase positive staphylococci presented by patients on admission 76 per cent were resistant to some antibiotic (37 per cent to streptomycin, 21 per cent to chlortetracycline, 29 per cent to oxytetracycline, 8 per cent to chloromycetin, and 4 per cent to all four mentioned). A similar story at Seattle is shown in the contrast of data for August 1951 to April 1952 versus January to April 1953 by Kirby & Ahern (14). The percentage of resistant staphylococci isolated rose from 42 to 61 per cent to both chlortetracycline and oxytetracycline; and "virtually all" strains tested were resistant to penicillin and streptomycin (only four exceptions). Finland, Haight & Wilcox of Boston City Hospital also generalize (15) that about 75 per cent of staphylococcal isolates were resistant to penicillin, 25 per cent to chlortetracycline and 33 per cent to oxytetracycline. They also have encountered erythromycin resistant strains in two cases of chronic infections. Similar studies have been made at the Chicago Municipal Contagious Disease Hospital by Lepper *et al.* (16), at the Bronx Hospital [Weil & Stempel (1)], and at the Veterans Administration Hospital, Hines, Ill. [Jackson, Vicher & Soska (17)]. As a result of *in vitro* experiments, Wright *et al.* (18) were able to generalize on the tendency for resistants to emerge in response to the most commonly used antibiotics in the following order: most rapidly to streptomycin→erythromycin →penicillin (except for streptococci which show little or no such tendency); regular but less high resistance to oxytetracycline; very little to chloramphenicol. The low incidence of chloramphenicol resistants in hospitals is confirmed by Needham & Nichols (12) and by Kirby & Ahern (14), who pointed out that the restricted use of chloramphenicol is at least in part responsible. Kirby & Ahern, however, noted a drop in chloramphenicol-resistant strains [20 per cent at the time it was in use in their hospital (August, 1951 to May 1952) to 6 per cent in 1953].

Shifting percentage of resistants to a given antibiotic is an interesting point, with bearing upon the significance of the whole problem. Lepper *et al.* (19) have reported such a set of data, covering five months in which erythromycin and chlortetracycline were in use (and penicillin not) followed by four months when penicillin and chlortetracycline were used (and erythromycin not). The results are summarized in Table II. The rise in resistance to erythromycin is spectacular. However, there was a drop in incidence after its use was discontinued; the same will be noted for penicillin, in the period when it was excluded. In either case a residual resistant flora persists, and it is interesting that the percentage falls to the same range for both antibiotics, about 30 to 35 per cent. Resistants to chlortetracycline, which was used throughout, rose within two months and stayed at about the same levels (about 60 to 80 per cent) with no tendency to rise further in the later months. In other words, the percentage of resistants to a given antibiotic bears some relation to the immediate past history of therapy in the hospital concerned. Such may be the explanation of differences in trends in different parts of the country. In Texas, Dukes & Dukes (20) recorded successively 36, 54, 63,

TABLE II

RELATIONSHIP OF SPECIFIC ANTIBIOTIC THERAPY TO APPEARANCE OF RESISTANT BACTERIAL STRAINS

	Time (Months)	Percentage resistants		
		Erythromycin	Chlortetracycline	Penicillin
Erythromycin Chlortetracycline Period	0	0	35	50
	1	6	40	45
	2	25	64	37
	3	50	66	32
	4	64	60	32
	5	70	80	35
Penicillin Chlortetracycline Period	6	33	60	60
	7	40	60	60
	8	33	75	61
	9	28	58	83

and 80 per cent of resistants to penicillin over the years from 1949 to 1953, and a corresponding increase from 16 to 32 per cent and from 5 to 40 per cent of resistants to chlortetracycline and oxytetracycline, respectively, in the years since their introduction. Weil & Stempel (1), on the other hand, while confirming the increase in resistants to penicillin, found no increase to chlortetracycline and oxytetracycline. However, their data for 1950-52 already showed resistance in the range of 30 to 35 per cent for both tetracycline antibiotics, and it may be that the resistants were stabilized at that level by the practices in the hospital concerned.

It is of interest to cite also the resistant flora in certain other countries as evidence that the problem is world wide. In Sweden, Lund (21) reported 76 per cent of all *Micrococcus pyogenes* var. *aureus* specimens received at the Statens Seruminstitut, Stockholm, to be penicillin-resistant. Only 13 per cent were resistant to streptomycin, perhaps reflecting less use of streptomycin in Sweden. Strains resistant to the newer antibiotics, neomycin, chlortetracycline, oxytetracycline, and chloramphenicol, ran from 7 to 15 per cent. In Scotland, Purdie (22) encountered 81 per cent of staphylococci resistant to penicillin from 1509 swab specimens.

Most of the above data on incidence of resistants concern hospital patients or critical infections referred by doctors, who had presumably already given treatments. They do not necessarily reflect a similar high incidence in the general public. For *M. pyogenes* some studies have been made of patients at admission, outpatients, and personnel of the hospital staff. Needham & Nichols (12) at Mayo Clinic reported 10 per cent carriers of resistant staphylococci in outpatients, and 20 per cent in apparently healthy

nurses. In the report of Ludlam (23), the evidence again points to the hospital personnel as carriers and donors of the resistant flora to the patients. In this report it is stated that 60 per cent of young infants harbored resistant staphylococci in the nares; in one particular hospital 100 per cent of the infants acquired them after from 1 to 10 days in the nursery; in this institution 87.5 per cent of resistant strains were found in 50 per cent of the nurses. After release of the infants from the hospital their resistant flora was lost, so that by the time they were one year old only 8.7 per cent were still carriers. Another study by Dowling, Lepper & Jackson (24) covers the data on 54 adult hospital patients at discharge and after some weeks at their own homes. At discharge 74 per cent of them carried strains of *M. pyogenes* var. *aureus* resistant to 10 units/ml. of penicillin, and 88 per cent carried strains resistant to 1+ units/ml. Resistant carriers in the hospital personnel were correspondingly frequent (51 per cent with strains resistant to 10 units, 85 per cent with strains resistant to 1+ unit). After four weeks at home the incidence of resistant strains in patients had dropped to 30 per cent and 36 per cent respectively, at which it remained as long as the study was carried, 21 weeks. Among contacts in the households, of whom 209 were studied, 30 per cent acquired resistant strains. The figure of 18 per cent carriers of penicillin-resistant strains was given by these authors for the public as a whole. Similar data for chlortetracycline were reported in the same paper.

Thus the problem of resistant flora in hospital patients is an acute one, which has grown more serious in spite of alternative antibiotics available for therapy. In fact, if it were not for new antibiotics discovered from time to time, the problem would be critical. At the moment erythromycin is saving lives (25) but there are already reports of acquired resistance (12, 14, 19).

In infections such as tuberculosis, it is particularly hard to avoid the development of resistant strains during the necessarily prolonged treatment (26). Combinations of antibiotics, or of antibiotics and drugs such as isoniazid and *p*-aminosalicylic acid, have been tried (27 to 33), in some cases with apparent success in delaying the appearance of resistant mycobacterial flora (27, 28, 29, 33, 34, 35). Combinations of antibiotics have also been used for many other infections, and have led to a whole new literature on cross resistance (36 to 39, and many more). As a result of the study of combinations of antibiotics or of antibiotics and other drugs, certain facts are beginning to emerge. According to Klein & Shorr (40) there are four possibilities resulting from interaction of antibiotics in the combinations:

Synergistic: combined effect of two drugs greater than that obtained by doubling the test concentration of either drug.

Additive: inhibiting effect of two drugs greater than that obtained with either but less than that obtained by doubling the concentration of either drug (an arbitrary Klett turbidity standard for growth inhibition by either drug alone was set up).

Interference: combined effect that is not greater than the inhibitory effect of either drug alone (within 10 points Klett).

Antagonism: combined effect less (at least 10 points Klett) than with either drug alone.

Closely related antibiotics like streptomycin and dihydrostreptomycin, and chlortetracyline and oxytetracyline show cross-resistance (13, 14, 18) and are additive. Synergism and antagonism are the more complex interrelations and are certainly not yet well understood, There are several reports of antagonisms in chemotherapy of tuberculosis by isoniazid and streptomycin (41, 42) or isoniazid and oxytetracycline (34); and in various infections by the combinations of penicillin and chlortetracycline (18, 43, 44), of penicillin and oxytetracycline (34), and of penicillin and erythromycin or carbomycin (45, 46) and viomycin (47). Gunnison *et al.* (48) have proposed certain groups of antibiotics: (*a*) Penicillin, streptomycin, bacitracin, neomycin; (*b*) Chloramphenicol, chlortetracyline, oxytetracyline. Mixtures of Groups *a* and *b* were said to be often synergistic, never antagonistic (but see 18, 34, 43, 44 above). Group *b* antibiotics were called additives. A much more elaborate study of cross resistance is found in the work of Szybalski & associates. In the first paper *Escherichia coli* reaction in resistance to 15 antibiotics (49), and in the second *Micrococcus pyogenes* var. *aureus* to 34 antibiotics and other drugs (50), were cross tested. The study of combined chemotherapeutic agents is very complex and often difficult to interpret. At times the effectiveness of combinations is based upon proportions of the chemotherapeutic agents (40, 41, 42), and there are numerous warnings that the dosage should be intelligently matched to the need, preferably after laboratory determination of the level of resistance of the patients' own flora (8, 17, 40, 45, 52). In the choice of antibiotics and of chemical additives to make them more efficient, future progress can be hoped for. With, in addition, intelligent dosage, according to newer knowledge of levels of the antibiotics in body tissues (31, 39, 48, 51, 52), clinicians of the future should be able to avoid some of the danger of acquired resistance of the pathogen in the infectious process.

Overgrowth by a Secondary Flora, Following Antibiotic Therapy

This topic has a significance which is only just beginning to be appreciated. The development of a secondary flora occurs, perhaps more often than we realize, in failures of antibiotics to cure a diseased host. The known cases are of at least three types and will have to be separately considered.

The most dramatic is the staphylococcal infection, in the form of enterocolitis (53 to 56) which is said to be a new disease entity in recent years. The condition has followed treatment with penicillin, streptomycin, chlortetracycline or oxytetracycline, but there is no evidence that they alone are responsible. It is characterized by a rapidly fulminating course with massive enteric infection and diarrhoea presenting nearly pure *M. pyogenes* culture. The disease develops in the hospital, in the postoperative patient or one with completely unrelated chronic infection such as rheumatic fever or infection

of the hip joint (54); in one case reported by Fairlie & Kendall (53) the antibiotic therapy had been intramuscular, and thus the "super-infection" of the intestinal tract cannot be considered a simple suppression of the normal intestinal flora permitting pathogenic overgrowth. A direct stimulus to the staphylococci was suggested, because in two patients the physicians merely withdrew the drug and the patients lived. For more positive therapy Dearing & Heilman (55) used erythromycin at 300 to 400 mg. four times daily with good results. Despite these reported staphylococcal accidents, one should not condemn antibiotic therapy as such. After all, one must remember the great incidence of *M. pyogenes* var. *aureus* in apparently healthy hospital personnel and even in infants and hospital patients who suffer no ill effects. An editorial in *Antibiotics & Chemotherapy* of June 1953 (7) suggests merely limiting dosage of the tetracyclines to avoid irritating the intestinal mucosa, and, if diarrhoea starts in a post-operative patient, prompt withdrawal of the antibiotic. Such is called the intelligent course, and if it is used there should be no need to ban the antibiotics concerned, as had been considered.

A somewhat similar occasional accident of antibiotic therapy is moniliasis (candidiasis) which is sometimes fatal (57, 58, 59). The cause of overgrowth of *Candida albicans* is not known. It was formerly found mostly in local lesions of the mouth, skin, nails, and vagina. One group of investigators (57) have commented that there seems to be a "noticeable increase" of fatal monilial infections following the oral use of antibiotics, especially the broad spectrum types and especially chlortetracycline. The danger is great enough that the Council of Pharmacy and Chemistry of the American Medical Association has recommended a statement on the label, warning of possible moniliasis. Is *C. albicans* merely an opportunist growing in the intestinal tract, in which normal flora is depressed by antibiotics? This is the suggestion of Miller (59), but it does not distinguish between the pathologic overgrowth of *C. albicans* and the general presence of it in hosts not apparently inconvenienced. McVay & Sprunt (60) reported that 63 per cent of the stools of some 186 patients on chlortetracycline yielded positive cultures of *C. albicans*, whereas they had been negative before therapy. It will be shown also in the next section of this review that yeasts may arise in great numbers in the intestinal tract of antibiotic-fed animals, which are in excellent nutritional state. To account for the pathologic moniliasis, therefore, other theories have been offered. Huppert, MacPherson & Cazin (58), Pappenport & Schnall (61), and Seligmann (62) and Werner (63) have shown a direct stimulation of *C. albicans* by chlortetracycline, *in vitro*. Moniliasis in mice has been produced also by oxytetracycline (64) and by all three broad spectrum antibiotics (65, 66).

Therapy for moniliasis was at first simple withdrawal of the antibiotic (53, 66, 67), but more recently specific chemotherapeutic agents such as fungicidin (64), candicidin (65), and undecylinic acid (66) have been advocated. For other yeast infections complicating antibiotic therapy, iodide in some form has been recommended, but these conditions may not be com-

parable to *C. albicans* infection. A blastomycosis of a tuberculous patient (68) and a yeast in bovine mastitis secondary to staphylococcal mastitis (69) have responded to iodide therapy.

Somewhat similar but less definite complications of overgrowth by previously harmless bacteria are known. Such genera as *Proteus* and *Pseudomonas* usually flourish in the intestinal tract when coliform bacteria and enterococci are depressed by administration of antibiotic (70, 71), and they or other gram-negative forms may at times invade the blood stream (72) or establish chronic renal or urinary infections (2, 72, 73, 74). Several types of antibiotic therapy of such infections are recommended: chlortetracycline, oxytetracycline, and chloramphenicol (73); polymyxin B alone (72, 74); or polymyxin B combined with oxytetracycline (2), chloramphenicol and neomycin (2), or penicillin and chloramphenicol (72). The combination of neomycin with phthalylsulfathiazole is claimed to be still better, since emergence of resistant strains is avoided (4), but it is hinted that bacitracin may have to be added if *Proteus* and *Pseudomonas* resistants appear.

In closing this section it is appropriate to mention one last topic of great concern to the medical profession in the use of antibiotics, namely, preoperative preparation for surgery on the gastrointestinal tract. Single antibiotics have, of course, been tried and the results judged by bacteriological analysis of the stools. Dihydrostreptomycin even when combined with sulfathaladine was called "far from satisfactory" by Riddell, Morton & Murray (75) because of irregular and temporary effect upon coliform flora, with reversion by the fifth day. Oxytetracycline also was unsatisfactory (71, 76). Doses as high as 3 gm. daily (750 mg. at six-hour intervals) can be tolerated by the patient, and, if given at the right time before operation, the coliform flora can be virtually eliminated; enterococci, *Proteus* and *Pseudomonas* spp. persist. However, the coliforms with yeasts and staphylococci gradually grow in numbers so that by the third to the fifth day the so-called "total aerobic" flora was as much as a hundred- to a thousandfold the original population level. Newer antibiotics have been tried and of them neomycin seems to offer the best control of intestinal flora in man. Alone it is said to give effective removal of aerobic flora (*Escherichia coli*, *Aerobacter aerogenes*, *Streptococcus faecalis*, *Proteus* and *Pseudomonas* spp.) but does not affect the anaerobes (*Bacteroides* and *Clostridium* spp.) in the experience of Dearing & Needham (3). Combined with oxytetracycline at the rate of 1 gm. neomycin and 0.25 gm. oxytetracycline every hour for four doses on the first day, then 1.5 gm. and 0.25 gm. four times on the second day, it gives "extremely effective" elimination of both aerobic and anaerobic intestinal flora (77). Poth (4) also claims neomycin to be quite effective and in combination with phthalylsulfathiazole even more so. Because the combination rests upon the novelty of neomycin (as yet few resistants have arisen), Poth suggests that it be kept for intestinal prophylactic use and asks the Council of Pharmacy and Chemistry of the American Medical Association to so restrict its use. Whether this is done or not, there are probably alternatives to

be found; one suggested by Marmell *et al.* (70) is said to be very good. It consists of formo-Cibazol alone or in combination with oxytetracycline. While this drug combination did not sterilize the tract ("no antibiotic or sulfonamide has yet succeeded in doing this"), it did so reduce the troublesome types, coliform, *Proteus*, staphylococci, and anaerobes, that there should be effective control of flora for prophylactic purposes.

Antibiotics in Animal Nutrition

Within the subject of the role of antibiotics in animal nutrition, we are interested for purposes of this review only in the changes in microbial flora of the intestinal tract. The influence of antibiotics on feed consumption efficiency, physiologic changes in the body, hatchability of eggs, phenomena of gestation or lactation, etc., will not be considered, despite their importance. Interested readers are referred to the excellent reviews of Stokstad (5) and Braude *et al.* (6) and to current literature as it appears, because at present these topics are being much discussed in terms of the explanation of the antibiotic effects.

At the outset it should be said that the antibiotic levels in feeding are very low in comparison to those for therapy. The recommended range is 2 to 50 p.p.m. in the feed, and the optima, as determined on chicks, are said to be 2 to 10 mg./lb. of feed for penicillin (78), 10 to 20 mg./lb. for chlortetracycline (79) and oxytetracycline (80). This is in the order of 0.5 to 2 per cent of that which, given by mouth, is required to develop a blood level in the range for therapy, and it is incalculably low compared to the levels that may be given to "sterilize" the intestinal tract (77). It is not surprising, then, to find that the feeding of antibiotics produces only temporary reductions in the flora and that they vary considerably with different animals, diets, and antibiotics. The principal work has been done with chicks, swine, and the laboratory rat, and more recently with ruminant animals, which present an entirely new problem of flora. In general the groups of microorganisms to be considered are the coliform, enterococci, lactobacilli, anaerobes (principally *Clostridium perfringens*), and yeasts. In preparing this review the writer has taken considerable trouble to chart the rise or fall of these groups in response to antibiotic feeding in the principal animals studied, but has not been able to arrive at any generalization, which is perhaps right in view of the controversy as to whether intestinal changes can explain the nutritional response or lack of it (5, 6, 81, 82, 83). There are many reports of an initial decrease in coliform count (for example, 84), followed by a later increase (82, 84 to 91), which may be significant because one of the theories of antibiotic effect is that intestinal synthesis of vitamins is stimulated. Such a secondary coliform flora has been shown to be antibiotic-resistant in some studies (82, 85). Since the coliform group may well be concerned in vitamin synthesis, tests have been made of the nutritional value of cells of *E. coli* and *A. aerogenes*. Anderson *et al.* (85, 86) fed cells of typical *E. coli*, atypical *E. coli*, and a mixture of many coliform organisms to chicks,

with and without penicillin. They obtained growth stimulation of the chicks fed on *E. coli* over controls, whether basal or fed on penicillin; chicks fed both *E. coli* and penicillin showed further increase. Romoser, Shorb & Combs (92), also working with chicks and with pure cultures of *E. coli* and *A. aerogenes*, confirmed the findings.

The *Lactobacillus* group and the enterococci may be considered together as lactic acid bacteria. They are known to require rather than synthesize vitamins, and it has been speculated that their presence in the intestinal tract robs the host of part of the dietary vitamin supply. They do not, however, respond in the same way to antibiotic feeding. The population of lactobacilli may decrease (82, 83), increase (87, 89) or remain uninfluenced (80, 88). The population of enterococci is generally increased (82, 89, 90, 91). The significance of the difference is not apparent.

The importance of the anaerobic group is also controversial, but for a different reason. Intestinal anaerobes undoubtedly comprise several species, both saccharolytic and putrefactive, but the dominant type, according to reports of the nutritional literature, is *C. perfringens*. It is, of course, a pathogen, and, in fact, produces a group of enterotoxins. One theory of the antibiotic effect is therefore that its elimination relieves the animal of toxemia, and thereby increases its well-being. Sieburth *et al.* (93) reported a sharp decrease in *C. perfringens* in turkeys administered either penicillin or oxytetracycline. The same has been found for rats (83, 88), but it may be influenced by the carbohydrate fed; with dextrin the number of fecal clostridia rises as much as three hundredfold (87, 94). In this respect the rat differs from other animals, for there is recent confirmation of Sieburth's finding that in turkeys the clostridia decrease in numbers (88, 91, 95). The significance of fluctuation in anaerobic counts is questioned by Larson & Carpenter (96), however, because there seems to be no correlation with growth response. It should perhaps be pointed out that such fluctuations, unrelated to growth phenomena, are perhaps to be expected from the very fact that different levels of antibiotic, as well as different kinds and combinations of antibiotics, are being fed. In exploring whether levels of antibiotics in the tract reach inhibitory values Yasowitz & Bird (97) have traced chlortetracycline, oxytetracycline, chloramphenicol, and penicillin given in single dose of 40 mg. to chicks. They record data for the proventriculus, gizzard, duodenum, upper and lower small intestine, cecum, cloaca, urine, and feces, and the gist of evidence is that except for chloromycetin there is still ample antibiotic in the cecum and below to have an effect. Chloramphenicol, which is the low level drug, is the only one to which growth response is not given by the chick. Barber *et al.* (94) have similar data for chlortetracycline and penicillin in the pig.

Correlation of other factors with growth response has likewise been elusive, and thus the literature of antibiotic feeding is difficult reading. There are reports on lack of growth response (98) or irregular growth response (99), on the effect of "new quarters" (100) versus "infected quarters" (101),

on the level and kind of protein (89, 94, 102), on diets deficient in methionine (103, 104) or other amino acids (105), on diets deficient in vitamins (98, 106 to 109), on carbohydrates and yeast in the diet (110), on oral versus parenteral route for administration (111, 112), on physiologic changes of the body and their significance (113 to 117), on certain antibiotics of little or no value (118, 119), and on control of enteric disease complicating the problem (94, 120). Perhaps related to the disease phenomenon is the fact that antibiotics prolong the lives of animals on the so-called necrogenic diet (121). Work on the intestinal flora here also shows many of the above changes.

Several theories, besides the theory of vitamin synthesis, have emerged to explain the effect of antibiotics on growth. These theories are well discussed in the reviews by Stokstad (5) and Braude *et al.* (6) and need not be repeated here.

Lastly, the relation of antibiotics to the nutrition of ruminant animals remains to be discussed. This is also a confusing subject at present. The ruminant relies upon the microbial population of the rumen for the decomposition of the plant tissue which it ingests. Consequently, any lethal effects that antibiotics might have could be expected to be detrimental to the animal; but perhaps in the mixed flora there are always forms to take over the functions of those eliminated. Nevertheless, imbalance might have profound effects. The microflora of the rumen itself is not well known because many of the microorganisms which are seen microscopically cannot as yet be cultured. The most recent thorough study of cultural methods and a new attempt to characterize groups of bacteria in the rumen is that of Bryant & Burkey (122, 123). However, only about 8.4 per cent of the flora seen by direct count was cultivated. Thus it seems rather futile to discuss changes of the flora under antibiotic influence. However, a few papers may be cited to show the trend of research. Three reports of feeding penicillin to calves (124, 125, 126) show negative or even detrimental effect upon growth. With chlortetracycline there are three positive reports on growth (126, 127, 128) and one negative (129). However, remarks upon the health of the calves, lack of scours, and early adaptation to hay and grain may favor the use of at least chlortetracycline for calves. With adult cattle a study has been made on fistulated steers to follow the passage of digested food out of the rumen (130), changes in rumen flora (131), and amino acid content of the rumen (132). On lactating dairy cows a study was made, again with chlortetracycline at 40 gm./1000 lbs. body wt. No benefit and no harm resulted (133).

Conclusion

When the antibiotics first offered a means of selective control of the microflora of the animal body, they were exploited in the treatment of diseases, often with spectacular success. Later as their use became widespread, difficulties appeared in the secondary effects upon the flora, either through induced resistance or imbalance of natural, previously minor groups within the flora. Like inflation, the tendency was to use more and newer antibiotics,

and so it is today that we find ourselves in the position that we cannot stop. New tricks of dosage or of combination of antibiotics or of derivatives more stable in the body are the present solution. But what of the future? Surely the use and abuse of chemotherapy lead to extremely complex phenomena. Who could have foreseen the value of antibiotics in animal nutrition? And what in future will there be in the way of new problems or new benefits, as antibiotics are applied to seeds, soil and other nonmedical uses? Future reviews will have no lack of material but what it may be we cannot foretell.

LITERATURE CITED

1. Weil, A. J., and Stempel, B., *Antibiotics & Chemotherapy*, **3**, 1135–40 (1953)
2. Erwin, C. P., Waisbren, B. A., and Kruse, R., *Am. J. Med. Sci.*, **226**, 525–32 (1953)
3. Dearing, W. H., and Needham, G. M., *Proc. Staff Meetings Mayo Clinic*, **28**, 502–7 (1953)
4. Poth, E. J., *J. Am. Med. Assoc.*, **153**, 1516–21 (1953)
5. Stokstad, E. L. R., *Physiol. Revs.*, **34**, 25–51 (1954)
6. Braude, R., Kon, S. K., and Porter, J. W. G., *Nutrition Abstrs. & Revs.*, **23**, 473–95 (1953)
7. Editorial, *Antibiotics & Chemotherapy*, **3**, 561–70 (1953)
8. Gould, J. C., Bowie, J. H., and Cameron, J. D. S., *Lancet*, **I**, 361–64 (1953)
9. Ainsworth, G. C., and Austwick, P. K. C., *Vet. Record*, **65**, 166 (1953)
10. Editorial, *Vet. Record*, **65**, 529 (1953)
11. Porter, J. W. G., *Vet. Record*, **65**, 288–90 (1953)
12. Needham, G. M., and Nichols, D. R., *J. Lab. Clin. Med.*, **41**, 150–56 (1953)
13. Miyahara, B. T., Cariker, K., and Clapper, W. E., *J. Lab. Clin. Med.*, **41**, 550–58 (1953)
14. Kirby, W. M. M., and Ahern, J. J., *Antibiotics & Chemotherapy*, **3**, 831–35 (1953)
15. Finland, M., Haight, T. H., Wilcox, C., and Broderick, M. K., *Arch. Internal Med.*, **91**, 143–58 (1953)
16. Lepper, M. H., Dowling, H. F., Jackson, G. G., and Hirsch, M. M., *Arch. Internal Med.*, **92**, 40–50 (1953)
17. Jackson, G. G., Vicher, E. E., and Soska, J. W., *Am. J. Clin. Pathol.*, **23**, 297–302 (1953)
18. Wright, S. S., Purcell, E. M., Wilcox, C., Broderick, M. K., and Finland, M., *J. Lab. Clin. Med.*, **42**, 877–95 (1953)
19. Lepper, M. H., Dowling, H. F., Jackson, G. G., Moulton, B., and Spies, H. W., *J. Lab. Clin. Med.*, **42**, 832 (1953)
20. Dukes, C. D., and Dukes, A. M., *Bact. Proc.*, 64 (1953)
21. Lund, E., *Acta Pathol. Microbiol. Scand.*, **33**, 393–400 (1953)
22. Purdie, I. A., *Lancet*, **I**, 952 (1953)
23. Ludlam, G. B., *J. Hyg.*, **51**, 64–74 (1953)
24. Dowling, H. F., Lepper, M. H., and Jackson, G. G., *Am. J. Public Health* **43**, 860–68 (1953)
25. Anderson, E. A., *Antibiotics & Chemotherapy*, **3**, 1091–94 (1953)
26. Mahady, S. C. F., Armstrong, F. L., Beck, F., Horton, R., and Lincoln, N. S., *Am. Rev. Tuberc.*, **68**, 238–48 (1953)

27. MRC. Isoniazid Trial: Rept. No. 4, *Lancet*, **II**, 217–23 (1953)
28. Karlson, A. G., and Feldman, W. H., *Am. Rev. Tuberc.*, **68**, 575–82 (1953)
29. Hobby, G. L., Lenert, T. F., Rivoire, Z. C., Donikian, M., and Pikula, D., *Am. Rev. Tuberc.*, **67**, 808–27 (1953)
30. Cohen, S. S., Johnson, L., Lichtenstein, M. R., and Lynch, W., *Am. Rev. Tuberc.*, **68**, 229–37 (1953)
31. Hobby, G. L., and Lenert, T. F., *Am. Rev. Tuberc.*, **68**, 292–98 (1953)
32. Mount, F. W., and Ferebee, S. H., *Am. Rev. Tuberc.*, **67**, 108–13 (1953)
33. Dye, W. E., Lynch, H. P., and Brees, A. G., *Am. Rev. Tuberc.*, **67**, 106–7 (1953)
34. Mackaness, G. B., and Smith, N., *Am. Rev. Tuberc.*, **67**, 322–40 (1953)
35. Wolinsky, E., and Steenken, W., Jr., *J. Bact.*, **66**, 229–37 (1953)
36. Vicher, E. E., Soska, J. W., and Jackson, G. G., *Arch. Surg.*, **66**, 283–91 (1953)
37. Fusillo, M. H., Noyes, H. E., Pulaski, E. J., and Tom, J. Y. S., *Antibiotics & Chemotherapy*, **3**, 581–86 (1953)
38. Fusillo, M. H., and Kuhns, D. M., *U. S. Armed Forces Med. J.*, **4**, 1600–5 (1953)
39. Dowling, H. F., Lepper, M. H., and Jackson, G. G., *J. Am. Med. Assoc.*, **151**, 813–15 (1953)
40. Klein, M., and Schorr, S. E., *J. Bact.*, **65**, 454–65 (1953)
41. Grunberg, E., and Schnitzer, R. J., *Am. Rev. Tuberc.*, **68**, 277–79 (1953)
42. Szybalski, W., and Bryson, V., *Am. Rev. Tuberc.*, **68**, 280–83 (1953)
43. Lepper, M. H., Jackson, G. G., Dowling, H. F., and Seto, J., *Am. J. Med. Sci.*, **225**, 648–56 (1953)
44. Jackson, G. G., Lepper, M. H., Seto, J., and Dowling, H. F., *Am. J. Med. Sci.*, **225**, 525–34 (1953)
45. Finland, M., and Wilcox, C., *Proc. Soc. Exptl. Biol. Med.*, **83**, 605–8 (1953)
46. Purcell, E. M., Wright, S. S., and Finland, M., *Proc. Soc. Exptl. Biol. Med.*, **82**, 124–31 (1953)
47. Manten, A., and Van Wijnagaarden, L. J., *Antonie Van Leeuwenhoeck J. Microbiol. Serol.*, **19**, 93–99 (1953)
48. Gunnison, J. B., Shevky, M. C., Bruff, J. A., Coleman, V. R., and Jawetz, E., *J. Bact.*, **66**, 150–58 (1953)
49. Szybalski, W., and Bryson, V., *J. Bact.*, **64**, 489–99 (1952)
50. Szybalski, W., *Antibiotics & Chemotherapy*, **3**, 1095–1103 (1953)
51. Kohn, K. H., Milzer, A., and MacLean, H., *J. Am. Med. Assoc.*, **151**, 347–51 (1953)
52. Meleney, F. L., and Johnson, B. A., *J. Am. Med. Assoc.*, **153**, 1253–60 (1953)
53. Fairlie, C. W., and Kendall, R. E., *J. Am. Med. Assoc.*, **153**, 90–94 (1953)
54. Gardner, D. L., *Lancet*, **II**, 1236–38 (1953)
55. Dearing, W. H., and Heilman, F. R., *Proc. Staff Meetings Mayo Clinic*, **28**,121–34 (1953)
56. Terplan, K., *Am. J. Pathol.*, **29**, 595–96 (1953)
57. Brown, C., Jr., Propp, S., Guest, C. M., Beebe, R. T., and Early, L., *J. Am. Med. Assoc.*, **152**, 206–7 (1953)
58. Huppert, M., MacPherson, D. A., and Cazin, J., *J. Bact.*, **65**, 171–76 (1953)
59. Miller, C. P., *Ann. Internal Med.*, **35**, 763–70 (1951)
60. McVay, L. V. Jr., and Sprunt, D. H., *Proc. Soc. Exptl. Biol. Med.*, **78**, 759–61 (1951)
61. Pappenport, R. B., and Schnall, E. S., *Arch. Internal Med.*, **88**, 729–35 (1951)

62. Seligmann, E., *Proc. Soc. Exptl. Biol. Med.*, **83**, 778–81 (1953)
63. Werner, G. H., *Lancet*, **II**, 676 (1953)
64. Hazen, E. L., Brown, R., and Mason, A., *Antibiotics & Chemotherapy*, **3**, 1125–28 (1953)
65. Kligman, A. M., and Lewis, F. S., *Proc. Soc. Exptl. Biol. Med.*, **82**, 399–404 (1953)
66. Mountain, D. C., and Krumenacher, F. P., *Am. J. Med. Sci.*, **225**, 274–80 (1953)
67. Gray, J. D., *J. Hyg.*, **51**, 322–29 (1953)
68. Stillians, A. W., and Klemptner, H. E., *J. Am. Med. Assoc.*, **153**, 560–61 (1953)
69. Steele-Bodger, A., *Vet. Record*, **65**, 304 (1953)
70. Marmell, M., DiLorenzo, J. C., Wright, L. T., and Metzger, W. I., *Antibiotics & Chemotherapy*, **3**, 1129–34 (1953)
71. Brottman, S. A., Cohen, J. R., and Litsky, W., *Am. J. Med. Sci.* **226**, 419–24 (1953)
72. Spink, W. W., *J. Am. Med. Assoc.*, **152**, 585–90 (1953)
73. Eisenberg, G. M., Alexander, J. D., Jr., and Flippin, H. F., *J. Am. Med. Assoc.*, **152**, 1302–4 (1953)
74. Hopper, J., Jr., Jawetz, E., and Hinman, F., Jr., *Am. J. Med. Sci.*, **225**, 402–9 (1953)
75. Riddell, M. I., Morton, H. S., and Murray, E. G. D., *Am. J. Med. Sci.*, **225**, 535–46 (1953)
76. Morton, H. S., Riddell, M. I., and Murray, E. G. D., *Am. J. Med. Sci.*, **225**, 646–47 (1953)
77. Dearing, W. H., and Needham, G. M., *Proc. Staff Meetings Mayo Clinic*, **28**, 507–12 (1953)
78. Heuser, G. F., and Norris, L. C., *Poultry Sci.*, **31**, 857–62 (1952)
79. Oleson, J. J., Hutchings, B. L., and Whitehill, A. R., *Arch. Biochem.*, **29**, 334–38 (1950)
80. Rosenberg, M. M., Morikawa, W., and Bushnell, O. A., *Poultry Sci.*, **31**, 708–14 (1952)
81. Branion, H. D., Anderson, G. W., and Hill, D. C., *Poultry Sci.*, **32**, 335–47 (1953)
82. Johansson, K. R., Peterson, G. E., and Dick, E. C., *J. Nutrition*, **49**, 135–52 (1953)
83. Scaletti, J. V., Kulp, W. L., and Cleverdon, R. C., *Proc. Soc. Exptl. Med.*, **83**, 599–602 (1953)
84. Moore, P. R., Evenson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, **165**, 437–41 (1946)
85. Anderson, G. W., Slinger, S. J., and Pepper, W. F., *J. Nutrition*, **50**, 35–46 (1953)
86. Anderson, G. W., Slinger, S. J., Pepper, W. F., and Hauser, M. M., *J. Nutrition*, **50**, 47–57 (1953)
87. Peterson, G. E., Dick, E. C., and Johansson, K. R., *J. Nutrition*, **51**, 171–89 (1953)
88. Guzman-Garcia, J., Sarles, W. B., and Baumann, C. A., *J. Nutrition*, **49**, 647–56 (1953)
89. Anderson, G. W., Cunningham, J. D., and Slinger, S. J., *Can. J. Comp. Med. Vet. Sci.*, **17**, 83–91 (1953)
90. Quinn, L. Y., Story, C. D., Catron, D. V., Jensen, A. H., and Whalen, W. M., *Antibiotics & Chemotherapy*, **3**, 527–33 (1953)

91. Quinn, L. Y., Lane, M. D., Ashton, G. C., Maddock, H. M., and Catron, D. V., *Antibiotics & Chemotherapy*, **3**, 622–28 (1953)
92. Romoser, G. L., Shorb, M. S., and Combs, G. F., *Proc. Soc. Exptl. Biol. Med.*, **83**, 17–21 (1953)
93. Sieburth, J. M., Gutierrez, J., McGinnis, J., Stern, J. R., and Schneider, B. H., *Proc. Soc. Exptl. Biol. Med.*, **76**, 15–18 (1951)
94. Barber, R. S., Braude, R., Kon, S. K., and Mitchell, K. G., *Brit. J. Nutrition*, **7**, 306–19 (1953)
95. Elam, J. F., Jacobs, R. L., Tidwell, W. L., Gee, L. L., and Couch, J. K., *J. Nutrition*, **49**, 307–17 (1953)
96. Larson, N. L., and Carpenter, L. E., *Proc. Soc. Exptl. Biol. Med.*, **79**, 227–29 (1952)
97. Yasowitz, H., and Bird, O. D., *Poultry Sci.*, **32**, 966–68 (1953)
98. Briggs, G. M., Spivey, M. R., and Ortiz, L., *Federation Proc.*, **12**(1346), 410 (1953)
99. King, J. O. L., and White, E. G., *Vet. Record*, **65**, 149–52 (1953)
100. Hill, C. H., and Kelly, J. W., *J. Nutrition*, **51**, 463–66 (1953)
101. Wilson, J. E., *Vet. Record*, **65**, 633–39 (1953)
102. Reynolds, W. M., Pendergrass, G. P., Appel, P. P., Luther, H. G., and McGinnis, J., *Federation Proc.*, **12**(1406), 427 (1953)
103. Atkinson, R. L., Reid, B. L., Quisenberry, J. H., and Couch, J. R., *J. Nutrition*, **51**, 53–64 (1953)
104. Hartsook, E. W., and Johnson, B. C., *J. Nutrition*, **51**, 205–18 (1953)
105. Pecora, L., *J. Nutrition*, **49**, 621–29 (1953)
106. Ham, W. E., and Scott, K. W., *J. Nutrition*, **51**, 423–33 (1953)
106a. Guggenheim, K., Halevy, S., Hartmann, I., and Zamir, R., *J. Nutrition*, **50**, 245–53 (1953)
106b. Chow, B. F., Davis, R. L., and Davis, S., *J. Nutrition*, **49**, 657–68 (1953)
107. Briggs, G. M., Spivey, M. R., and Ortiz, L. O., *J. Nutrition*, **51**, 355–64 (1953)
108. Rhodes, R. A., Sarles, W. B., Monson, W. J., Harper, A. E., and Elvehjem, C. A., *J. Nutrition* (In press)
109. Monson, W. J., Harper, A. E., Winje, M. E., Elvehjem, C. A., Rhodes, R. A., and Sarles, W. B., *J. Nutrition* (In press)
110. Stokstad, E. L. R., Jukes, T. H., and Williams, W. L., *Poultry Sci.*, **32**, 1054–58 (1953)
111. Terrill, S. W., Becker, D. E., Gard, D. I., and Lassiter, J. W., *Antibiotics & Chemotherapy*, **3**, 1011–14 (1953)
112. Fell, R. V., and Stephenson, E. L., *Poultry Sci.*, **32**, 1092–93 (1953)
113. Teh-Cheng Huang, and McCoy, C. M., *J. Nutrition*, **50**, 129–39 (1953)
114. Catron, D. V., Lane, M. D., Quinn, L. Y., Ashton, G. C., and Maddock, H. M., *Antibiotics & Chemotherapy*, **3**, 571–77 (1953)
115. Dymsza, H., Boucher, R. V., and Callenbach, E. W., *Poultry Sci.*, **32**, 989–93 (1953)
116. Braude, R., and Johnson, B. C., *J. Nutrition*, **49**, 505–12 (1953)
117. Maddock, H. M., Catron, D. V., Spier, V. C., Cuff, P. W. W., and Ashton, G. C., *Antibiotics & Chemotherapy*, **3**, 1075–80 (1953)
118. Braude, R., Wallace, H. D., and Cunha, T. J., *Antibiotics & Chemotherapy*, **3**, 271–91 (1953)
119. Becker, D. E., Terrill, S. W., Lassiter, J. W., Nelson, T. S., and Gard, D. I., *Antibiotics & Chemotherapy*, **3**, 778–82 (1953)

120. Rossoff, I. S., and Abel, W., *Vet. Med.*, **48**, 369–70 (1953)
121. Forbes, M., Shockman, G. D., Chu, E., and György, P., *Antibiotics & Chemotherapy*, **3**, 1104–10 (1953)
122. Bryant, M. P., and Burkey, L. A., *J. Dairy Sci.*, **36**, 205–17 (1953)
123. Bryant, M. P., and Burkey, L. A., *J. Dairy Sci.*, **36**, 218–24 (1953)
124. Knodt, C. B., and Ross, E. B., *Proc. Soc. Exptl. Biol. Med.*, **82**, 663–65 (1953)
125. Hibbs, J. W., Conrad, H. R., and Pounden, W. D., *J. Dairy Sci.*, **36**, 1319–25 (1953)
126. Knodt, C. B., *Antibiotics & Chemotherapy*, **3**, 442–48 (1953)
127. Knodt, C. B., Ross, E. B., and Stein, J. F., *J. Dairy Sci.*, **36**, 1201–4 (1953)
128. Bartley, E. E., Fountaine, F. C., Atkeson, F. W., and Fryer, H. C., *J. Dairy Sci.*, **36**, 103–11 (1953)
129. Bloom, S., and Knodt, C. B., *J. Dairy Sci.*, **36**, 633–36 (1953)
130. Chance, C. M., Huffman, C. F., and Duncan, C. W., *J. Dairy Sci.*, **36**, 253–59 (1953)
131. Chance, C. M., Duncan, C. W., Huffman, C. F., and Luecke, R. W., *J. Dairy Sci.*, **36**, 495–503 (1953)
132. Chance, C. M., Smith, C. K., Huffman C. F., and Duncan, C. W., *J. Dairy Sci.*, **36**, 743–51 (1953)
133. Bartley, E. E., Fountaine, F. C., Atkeson, F. W., *J. Dairy Sci.*, **36**, 402–8 (1953)

PHYSIOLOGICAL ASPECTS OF PROTOZOAN INFECTION[1]

By B. G. Maegraith

Professor of Tropical Medicine, Liverpool School of Tropical Medicine, Liverpool, England

MALARIA

THE HOST AND THE PARASITE

The pathogenic agent in malaria is generally considered to be the erythrocytic (E) parasite. Apart from local damage resulting directly from invasion, tissue forms of parasite, at any rate in mamalian malaria, are probably non-pathogenic. In this review the activity of the E form only is considered.

The estimation of the metabolic and nutritional requirements of the malaria parasite is technically difficult because of its highly specialized environment within the red cell, which makes it difficult to distinguish between the metabolism of the parasite and that of the cell containing it, especially when the later is nucleated, as in avian infections, or immature, as in berghei malaria. At the moment, moreover, there is no way of determining whether the presence of the growing parasite alters the metabolic behavior of the host cell and other tissues. Nevertheless, some information is available regarding the parasite which indicates that, despite its specialized intracellular nature, its nutrient requirements and metabolic pathways (except for the metabolism of glycerol, the splitting of haemoglobin, and the failure to synthesize glycogen) are broadly similar to those of the host (1). It is possible, therefore, that direct competition between parasite and host might occur. There is, however, little evidence of this, although the development of the parasite can be considerably influenced by changes in the host diet (2 to 7).

THE BLOOD OF THE HOST

Erythrocytes.—Some of the changes in erythrocytes in mammalian malaria are attributable to the type of cell invaded. For instance, *Plasmodium vivax* and *P. berghei* both show predilection (in the latter case almost complete) for immature cells, which probably accounts for the reported enlargement of invaded cells in these infections. There is very little evidence of change of erythrocyte volume in malaria.

The surface electrical charge of parasitized and unparasitized erythrocytes is reduced during avian infections contemporaneously with the appear-

[1] This review covers certain protozoan infections. For reasons of space only those attributable to *Plasmodia* and *Entamoeba* are discussed in any detail. The effects of infection with *Trypanosoma*, *Leishmania*, and *Babesia* are considered in relation to the above in a general summary. The bibliography has been kept to very restricted limits by making reference wherever possible to reviews and books. The survey of the literature pertaining to this review was completed in November, 1953.

ance of humoral immune bodies, and the erythrocyte sedimentation rate is commonly increased in all infections, especially when the anaemia is severe. In mammalian malaria there is either no change or a decrease in saline fragility; increased fragility to lysolecithin has been reported (8, 11). Changes in the properties of the surface of unparasitized erythrocytes leading to redistribution of electrolytes between unparasitized cells and plasma have been noted.

Certain mechanical effects, such as the accumulation of parasitized cells along the vascular endothelium and the formation of sludge (9), occur in the circulation of the blood in malaria and may be a result of changes in the erythrocytes. The specific increase in phagocytosis of both parasitized and unparasitized erythrocytes which develops during malaria, particularly in the spleen, may also involve some change in the red cells.

The total haematin content of the infected cell remains constant in mammalian malaria during the growth of the parasite. The content of haemoglobin falls, however, as the pigment is broken down and the insoluble haematin-containing haemozoin is produced. Other pigments such as methaemoglobin have not been detected in either parasitized or unparasitized cells.

The respiratory function of the unchanged haemoglobin in the parasitized mammalian cell and of that in the unparasitized cell is not appreciably altered: samples of blood from infected animals usually take up and discharge oxygen normally *in vitro* so long as the cell environment is kept within physiological limits. In ducks infected with *P. lophurae* a shift of the oxyhaemoglobin dissociation curve to the right has been noted, associated with a fall in alkali reserve. This has been interpreted as indicating inefficient oxygen transport by the blood, but deviation of the curve to the right should in fact facilitate oxygen transfer at low tensions (10).

It thus appears that the oxygen carrying capacity of blood during malaria is probably, as in normal blood, a function of the total haemoglobin content. There is no indication of serious competition for oxygen between tissues and parasite (11, 12).

Both infected and unparasitized erythrocytes are destroyed during an attack. The cause of the haemolysis is unknown. Erythrocytes from complicated falciparum malaria have been stated to be more labile than normal cells when injected into a normal circulation, which suggests there may be some change in the cells themselves which renders them more liable to lysis (13). On the other hand, the cell environment in malaria may be at fault. Animal tissues have been shown to possess lytic properties which are inhibited by normal plasma. It has been suggested that lysis may result from inhibition of the factors which themselves normally inhibit this tissue lytic effect, which Ponder has suggested may be attributable to lysolecithin.

A lytic substance, believed to be an unsaturated monocarboxylic fatty acid, has recently been demonstrated in normal human serum and infected monkey erythrocytes, and it has been suggested that this may accumulate in

the parasitized cell and be responsible for its rupture at sporulation (14).

There is no evidence of the accumulation *in vivo* in parasitized cells or plasma of products of incomplete metabolism, such as lactic acid. Soluble lysins or variations in the physicochemical environment of the erythrocyte which might be important in haemolysis, have not been demonstrated. The malaria pigment liberated at sporulation is regarded as inert. It has been suggested that haemolysis may be the outcome of antigen-antibody reactions. It is possible, for instance, that the parasite may make the invaded erythrocyte autoantigenic (8).

The bone marrow response in mammalian malaria is essentially normoblastic and is reflected in a normocytic, sometimes slightly macrocytic blood picture. The mean haemoglobin content of erythrocytes may be normal or reduced.

Changes in the blood.—A raised level of plasma potassium might be expected as the result of erythrocyte destruction in malaria and has frequently been found, in both mammalian and avian infections. There is probably some relation between the rate of cell destruction and the prevailing level of plasma potassium, since the maximal concentration of potassium coincides with the period of maximal sporulation in human and monkey malaria. Nevertheless, the high concentrations recorded cannot be explained entirely in terms of haemolysis. Overman (18) recently confirmed this by demonstrating a shift of potassium from the uninfected erythrocyte to the plasma, associated with a movement of sodium into the cells. He found there were only relatively minor corresponding changes in plasma electrolytic concentrations so long as kidney function remained normal. Other workers have recorded reductions in both plasma sodium and chloride.

Deviations in renal function and loss of fluid and electrolytes from sweating, vomiting, and diarrhoea also influence the picture. Both increase and decrease in inorganic phosphorus content of the blood have been reported in mammalian infections. The very considerable increase in total concentration of phosphorus, resulting from synthesis of organic phosphorus compounds in the erythrocyte during parasite development, indicates that the parasite must accept phosphorus from outside the host cell. *In vitro* study of cell-free parasites has recently confirmed this (4, 15).

Most observers have found little change in alkali reserve in uncomplicated malaria; it may, however, be low during severe lysis (16).

Blood volume.—Overman (18) recorded an increase in thiocyanate space during the active stages of *P. vivax*, *P. falciparum*, and *P. knowlesi* infections, with progressive reduction in the total erythrocyte volume, usually offset by a roughly equivalent increase in plasma volume. An increase in plasma volume in active uncomplicated vivax and falciparum malaria has been noted by other workers, some of whom have also observed a slight overall reduction in total blood volume during the paroxysm. Dilution of the blood may be a contributory factor in the prevailing anaemia (17, 18, 19).

Pronounced reduction in blood volume in severe falciparum malaria has

frequently been recorded, as might be expected in a condition in which shock may develop. Such reduction may not always be obvious because of the coincident loss of circulating erythrocytes. With the fall in plasma volume there is a corresponding rise in haemoglobin concentration and red cell count, which may occur in individuals with minimal erythrocyte destruction (20).

Blood pigments.—Small amounts of free haemoglobin may appear in the plasma in various forms of malaria and much larger amounts in blackwater fever. Many authors have recorded the presence of "methaemoglobin" in the plasma in human and monkey malaria; but Fairley has shown that this pigment, which is produced only in primates, is not methaemoglobin but a firm compound of haematin and albumin which he has called methaem-albumin (21).

The fate of malarial pigment is not known, except that it is removed rapidly and stored by the macrophage-lymphocyte system. In avian malaria it may slowly become available for the resynthesis of haemoglobin, but nothing is known of its availability in mammalian malaria (22, 23). No investigation of iron metabolism in malaria seem to have been made. Rapid lysis and deviations in liver function may give rise to bile pigments in the plasma, associated with increase in their discharge in the urine.

Blood sugar.—The hyperglycaemia observed during the paroxysms in vivax malaria and in *P. lophurae* infection in well-fed ducks, but not in starving birds, is usually attributed to excessive liver glycogenolysis; possible factors concerned in which are increased adrenal activity, increased metabolic activity of fever, and damage to the hepatic cells.

Hypoglycaemia may occur in human malaria in cases in which infection persists for some time, and during severe *P. knowlesi* infections in *Macacus mulatta*, and in *P. lophurae* infection in ducks. This hypoglycaemia may be related to the glycogen stores of the liver, since in knowlesi infections Fulton found the lowest blood glucose values associated with the lowest values of liver glycogen, the latter being lower in infected animals than in controls (24, 25). There is no evidence that glycogen synthesis is affected in malaria except when liver function is disturbed.

Most observers consider that direct competition for glucose between the parasite and the host is inappreciable. On the other hand, there is some evidence thet the parasites may themselves be dependent on the available circulating glucose (2, 7, 26)

Protein.—In the active stages of mammalian malaria the total plasma protein often falls as a result of reduction in albumin. The albumin/globulin ratio is reversed, and there is frequently an absolute increase in globulin. These changes have been given some attention in view of their possible relation to antibody formation. The increase in mainly in the gamma globulin fraction. A gamma globulin with an inhibitory effect *in vitro* on *P. knowlesi* has been isolated from the plasma of superinfected monkeys. (2, 27)

Changes in protein pattern have been related to changes in the activity

of the reticulo-endothelial system and the liver, and to low protein intake or stores, especially in poorly nourished hosts. Despite the fact that the growing intracellular parasites require certain amino acids and other nitrogenous substances from the plasma, there is no evidence of competition between host and parasite. No estimate seems, however, to have been made of the total synthesis of protein by the parasites during an infection. In starved animals parasite development is inhibited even though the plasma content of methionine and *p*-aminobenzoic acid may be raised. The administration of methionine under these conditions restores growth of the parasite. McKee considers this to indicate that "the body's vital needs for the amino acid takes precedence over those of the parasite." (2, 4). Change in alpha and beta agglutinin plasma content and the presence of cold agglutinins have been recorded in human malaria.

Lipids.—The total blood lipid and cholesterol concentrations in both vivax and falciparum malaria commonly fall and return to normal after treatment. In severe *P. knowlesi* infections a fall of total cholestrol and total lipid has been noted 24 hr. before the onset of haemoglobinuria, followed by a sharp rise just before death. In animals which do not develop haemoglobinuria, the fall in cholesterol continues uninterrupted to death. No satisfactory explanation has been offered for these changes, which may possibly influence the prevailing lysis. Hypercholesterolaemia has been observed during the paroxysm in vivax malaria. This may be attributable to the altered fat metabolism of fever, but excessive destruction during the paroxysm of erythrocytes, in which synthesis of fatty substances is considerable, may also be a factor. No information is available regarding competition between host and parasite in the synthesis of fatty substances, although there is withdrawal of phosphorus from the plasma (4, 24).

Increase in biotin in the plasma of birds infected with *P. lophurae* and *P. cathemerium* has been noted; a decrease occurs in vivax infections. The *p*-aminobenzoic acid content of plasma increases considerably in knowlesi infections. It has been suggested that this may indicate mobilization from the tissues for use by the parasites; the fact that the concentration is raised in starvation contemporaneously with the inhibition of parasites does not however support this contention (2, 4).

General Effects

The febrile response and paroxysm.—The obvious time relation between parasitic sporulation and the appearance of the clinical paroxysm has not yet been explained. There is little evidence to connect the initiation of this reaction with the liberation into the circulation of merozoites, erythrocyte debris, protein, haemoglobin, or malaria pigment. The clinical features of the paroxysm are similar to those resulting from intravenous injection of pyrogenic agents and the humoral effects to those of anaphylactic shock. Most of the latter, however, can also be explained in terms of circulating hormones. The levels of plasma potassium reached are not regarded as toxic.

It has been suggested that the unknown initiating factor may be some non-specific pyrogenic agent liberated by the parasite (28).

Anaemia, anoxaemia, and tissue anoxia.—There is, little evidence to suggest that anoxaemia arising from anaemia is important in malaria except when the latter is extreme. Many of the most devastating effects of malaria may appear in hosts in which blood cell loss is minimal.

Incomplete oxygenation of the haemoglobin of circulating erythrocytes may occur as the result of changes in pulmonary circulation or the development of pulmonary oedema, or of intravascular corpuscular agglomeration, such as sludge. On the whole, these effects tend to be small, and it appears that the oxygen-carrying capacity of surviving erythrocytes is seldom lowered to the point of creating appreciably anoxic conditions at the tissues. Moreover, as noted above, the behaviour of infected blood *in vitro* suggests that oxygen dissociation at the tissue face probably proceeds satisfactorily (8, 10, 11, 23, 29).

Nevertheless, there is evidence that tissue anoxia of some kind develops in severe malaria, even when there is no obvious cause. It is possible that this anoxia may arise from interference with oxygen acceptance by the tissue cells (1, 28, 30).

Tissue changes.—Many of the physiological changes in the host during malarial infection arise from changes in individual organs. The pathological processes involved include general and local alterations in the circulation of the blood. Factors which influence the general circulation are fever and, in some forms of malaria, vascular failure or shock. Heart failure is uncommon except after continued high fever.

Variations in general peripheral circulation accompanied by fluctuations in blood pressure occur in the paroxysm, but these have little other than temporary effect on the host. Serious vascular disturbances, however, arise in certain infections causing effects (such as algid malaria) which are similar to those of medical shock (28, 31).

Many changes in the circulation through the various organs develop in conjunction with shock, and may have a profound effect on both the function and structure of the tissues concerned. These local circulatory changes which also appear in the absence of shock, and are often common to pathological states other than malaria, are significant factors in the initiation and maintenance of organ dysfunction. Dynamic circulatory disturbances commonly met include over-all reduction and redistribution of renal blood flow, which lead eventually to tubular damage and sometimes anuria (32, 33), and the redistribution of blood flow in the liver which results in congestion and centrilobular cellular changes (1, 34, 35). Phenomena of a different type occur in other organs, for example, the brain, in which the basic change is probably in endothelial permeability, giving rise to impedance of circulation from local loss of fluid (36). Mechanical interference with local circulation may also arise from changes in erythrocytes and from swollen and laden phagocytes either in the vessel walls or lying free in the lumen.

Haemorrhages into the perivascular tissues, such as seen in the brain in advanced falciparum malaria, are probably late developments. The same is true of thrombosis. It is not clear what part the presence of parasites play in these vascular changes, which are often associated with shock rather than with local accumulation of plasmodia, and may occur in relatively light infections (1, 28).

Changes in the local circulation in the endocrine glands may initiate general physiological effects, by altering the hormonal balance. There are, for instance, many features of an acute malarial attack which suggest adrenal involvement, even though pathological changes in the organ are by no means always present (1, 17, 18). Overmann (17) recently pointed out that the ionic anatomy of plasma and erythrocytes during an attack could be explained in terms of imbalance of the various adrenal hormones. There are, however, many other factors involved in water/salt balance which confuse the issue, including the effects of dehydration, vomiting, diarrhoea, changes in plasma volume, and disturbances of renal function and of carbohydrate metabolism.

The difficulty is to determine in any given case what part adrenal dysfunction (with or without local pathological changes) plays as a primary factor. Shock and associated phenomena very similar to those of adrenal insufficiency may arise in pernicious malaria independent of adrenal damage. Moreover, shock and tissue anoxia may produce pathological adrenal changes and cause humoral effects similar to those of adrenal insufficiency (28, 31). Adrenal functional derangements may thus in some instance be secondary to local circulatory changes. Humoral and cellular reactions are also important in determining the physiological response of the host to the infection. Changes in the spleen afford a good example of this.

Early in some forms of malaria, cellular hyperplasia occurs particularly amongst the cord cells and follicular lymphocytes. These changes are especially active during the crisis of self-limited malarial infections or after specific treatment, and are associated with a tremendous increase in phagocytosis, which is specific for the infecting strain of parasite and is regarded as evidence of the development of acquired resistance, which becomes manifest also by the appearance of humoral immune bodies. This acquired resistance may considerably modify the effects of the infection (37, 38).

In severe infections, and probably in all, there are also elements of degeneration and necrosis of unknown origin and not necessarily related to any local accumulation of parasites. These are prominent in most tissues in falciparum and knowlesi infections, in which, for instance, the hyperplastic reaction in the spleen is overwhelmed and a very considerable loss of lymphoid tissue and degeneration of cord cells takes place.

Further details of tissue lesions in malaria have been reviewed elsewhere and cannot be given here. The processes outlined above are all concerned in some degree in their evolution. It is generaly agreed that malarial pigment is not active in their development (39, 40).

AMOEBIASIS

Intestinal Amoebiasis

The invasion of the gut wall.—Considerable variation in virulence and pathogenicity has been reported in strains of *Entamoeba histolytica.* There seems to be little relation between the virulence of a strain (which is apparently an inherent quality) and its ability or otherwise to grow well *in vitro.* It is not certainly established whether *E. histolytica* in man is an obligate tissue pathogen or not; the erratic clinical course of infection suggests that it may alternate between pathogenicity and commensalism.

Many factors are involved in deciding the outcome of an amoebic infection, including the number of amoebae present, their opportunities of presentation at the epithelial surface, and their pseudopodial activity (41, 42). Long-continued cultivation *in vitro* decreases invasive powers, which can sometimes be restored by animal passage (43).

The outcome of an infection may sometimes be influenced by the bacterial flora of the intestinal contents (44, 45) and by the prevailing oxidation-reduction potential and pH, which may determine the region of the gut invaded (46).

The host diet may also influence the chances of tissue invasion, possibly by affecting the content of the large intestine. The literature on the effects of diet on amoebic infections has been recently reviewed by Taylor *et al.* (47, 48, 49). Mechanical injury to the gut wall may assist invasion, but it is generally agreed that *E. histolytica* can penetrate the undamaged epithelium, either by pseudopodial activity or by the action of lytic substances.

Histological evidence of epithelial lysis has frequently been described in amoebic infections but the immediate postmortem invasion of cells renders most of this evidence equivocal (50). Craig, however, demonstrated haemolysins (probably nonspecific) and tissue lysins in alcoholic extracts of amoebic cells (51, 52), and Morita reported destruction of epithelial cells after incubation with amoebae (53). Harinasuta has recently observed lysis of gelatin on photographic films by *E. histolytica* and by fresh extracts of amoebic cells (54).

Amoebae most commonly invade the intestinal epithelium in the interglandular areas but they may also enter the glands against the flow of mucus, which may be increased (55, 56). The likelihood of invasion also depends upon the host resistance, which may be affected by the diet. For instance, considerable enhancement of the invasive powers of *E. histolytica* in guinea pigs was demonstrated recently in vitamin C-deficient guinea pigs (57).

The invasion of intestinal tissues.—Once it has penetrated the epithelium *E. histolytica* readily invades the loose tissue of the gut wall. The principal barriers to further invasion are the muscular layers of the gut, especially the outer coat, and to a lesser extent the *muscularis mucosae.*

The mode of spread in the tissues is not understood. Migration may to some extent be aided by amoebic movements and by intestinal contractions,

but cytolysins are probably also involved. The integrity of the connective tissue may be challenged by lytic enzymes similar to hyaluronidase. *E. histolytica* grown in the usual culture media does not possess this enzyme, but suspensions of washed amoebae obtained from artificially produced liver lesions in hamsters contain it. Hyaluronidase may thus apparently be synthesized by amoebae under certain conditions. It remains to be determined whether the enzyme is present during host tissue invasion (58).

In infections of the natural host local cellular response is usually minimal. The reaction is not pyogenic, and the phagocytic response is slight. The cells called into play are mostly lymphocytic. Blood vascular changes are not usually pronounced, but there is some evidence of increased local vascular permeability. Inflammatory exudates may be present in established lesions in which there is secondary infection.

Tissue resistance to invasion is evidenced in some instances by the frequency of healing of small lesions or by the formation of granulation tissue at the base of the ulcers. Fibroblastic response is sometimes seen at the periphery. The mode of action of host resistance is not understood. The tissue may create conditions of pH and Eh inimical to the parasite. Evidence of the presence of humoral immune bodies also exists in established infections (50, 59, 60). Local immunity has been demonstrated in dogs in which treatment of intestinal infection may be followed by resistance to further infection with homologous and heterologous strains of *E. histolytica* (61).

Extraintestinal Amoebiasis

Spread of amoebic processes to other organs is regarded as secondary to their establishment, temporary or otherwise, in the intestinal tissues, and occurs either by contiguous extension or by metastasis via blood vessels or lymphatics. Extension to neighbouring tissues is the result of the progress of the invasive processes already outlined and is associated with existing active intestinal lesions. Granulomatous tissue such as amoebomata gradually build up as the processes of invasion, tissue destruction, and partial fibroblastic repair succeed one another.

No viscerotropic strain of *E. histolytica* has been demonstrated. It is presumed that the spread of infection to remote organs is metastatic, the original focus being in the intestine. Lesions in tissues other than the gut, although presumed to have arisen as a result of gut infection, are not always associated with contemporaneous active intestinal lesions.

Metastatic distribution of ameobae from intestinal lesions via blood vessels or lymphatics is regarded by some workers as commoner than is indicated by the incidence of remote lesions. This view is not accepted by others, but the chance of parasitic survival at the site of arrest is probably small. This is indicated by the frequent failure to produce metastatic lesions experimentally, and possibly by the rarity of amoebic lesions in abdominal lymph nodes.

Metastatic lesions occur most frequently in the liver, the lung, and the

brain in that order, the liver being by far the commonest site. Lesions in other organs are rare. Only the liver changes can be discussed here.

Hepatic amoebiasis in man.—Amoebic infection in the liver in man is clinically almost always associated with abscess formation. There is no good evidence of the existence of generalized or focal infection without abscess, such as occurs occasionally in experimental animal infections, although deviation in hepatic "function" tests have been observed in cases in which no involvement of the liver was clinically evident. Jaundice rarely develops even when abscess is present (62).

In man, overt abscesses are more commonly single than multiple, and occur in the right lobe about five times more frequently than in the left. This localization has been partly explained by the demonstration of streamlines in the portal venous blood flow (63).

The histological features of an abscess depend largely upon whether or not secondary bacterial infection has occurred. The contents of the uninfected abscess are sterile and composed of cellular debris. The liver tissue surrounding the lesion exhibits only light vascular and cellular reactions in which the predominant cells are lymphocytes and other mononucleate cells, including macrophages. The spreading edge of the abscess is composed of degenerate polygonal cells, amongst which amoebae are commonly found; there is minimal fibroblastic reaction.

How an abscess develops from metastatic amoebae is not known. There is some evidence that the initial lesion may be an infarct resulting from embolism of a small branch of the portal vein. Early lesions of this sort have been described, and emboli containing amoebae have been detected in the portal venous tree. The spread of the necrotic lesion into the liver tissue apparently occurs by direct extension. Small infarcted periportal necrotic lesions containing amoebae are common in guinea pigs within 24 hr. of injection of amoebae into the portal vein. As these experimental abscesses grow, satellite centrilobular degenerate lesions commonly develop at their periphery, suggesting an anaphylactic response. Similar centrilobular lesions have been described in human cases (64, 65). Amoebae are most easily recovered from early lesions and are much commoner in the abscess walls than in the contents.

Increase in size and weight of the liver is common in the presence of abscess and during the course of uncomplicated intestinal amoebiasis in human and animal infections. Amoebae have occasionally been recovered from the substance of the enlarged liver in animal but not human infections (66).

Some hold that the untreated abscess always progresses, others that healing with ultimate fibrous replacement of the abscess and regeneration of liver tissue may sometimes take place. There may be no obvious clinical relation between the activity of the intestinal lesion and the presence of an abscess, but the latter is rare in acute dysenteric cases, and much commoner in established cases during remission.

Experimental extraintestinal amoebiasis.—Abscesses have occasionally developed spontaneously in the course of experimental intestinal amoebiasis in animals. Attempts to produce ameobic liver abscess have been made in kittens, cats, dogs, hamsters, rats, and guinea pigs. Abscesses or "hepatitis" have been produced by injection of infective material into the liver substance in kittens, dogs, hamsters, and rats, but not by injection of amoebae into the mesenteric or portal veins (67 to 72). Recently, however, abscesses containing amoebae and associated bacteria have been produced and maintained by the inoculation of amoebae into the portal vein in guinea pigs in which long continued or "chronic" intestinal amoebic lesions had been set up (73). These experiments suggest that survival of metastatic amoebae reaching the liver may be prolonged by some sort of "preparation" of the tissues, possibly in the nature of sensitization.

The relation between the appearance of general symptoms and signs and the presence of the gut infection is not clear. Some authors believe that there is a continuous but irregular escape of amoebae from the intestinal lesion into the blood vessels or lymphatics. Others deny this.

No active product of amoebic metabolism or specific "toxin" has been discovered. On the other hand, since amoebic lesions in the gut are commonly secondarily infected, there may be a bacterial element in the general effects of intestinal amoebiasis (74).

GENERAL

The above brief reviews bring out certain general principles which appear to be common to other protozoan infections.

Some parasites have most of the metabolic processes of their hosts, others are deficient. Nevertheless, their sources of energy come from the host, so that it might be expected that under some circumstances the two could be forced into direct metabolic competition. There is, however, no good evidence of direct competition between parasites and host in any infection, except perhaps locally in individual cells wherein the parasite grows and multiplies (2, 4). For example, the invasion of the erythrocyte in malaria and babesiasis leads to local loss of oxygen, but in the body as a whole the oxygen carrying reserve of the uninfected cells remain more than adequate for essential tissue demands (11, 75, 76).

It is difficult to estimate the importance of the metabolic pattern of a particular parasite in relation to the host. There is little doubt, however, that it must determine the ultimate sites of invasion and development within the host. For instance, *E. histolytica* invades the epithelium of the gut in regions where the physico-chemical and nutritional conditions are most suited to its growth (46). The matter is more complicated in arthropod transmitted infections, but here again the particular phase of the parasites invades an environment which is suited to it. Thus the injected sporozoites develops in the tissue cell, the E form of the plasmodium in the erythrocyte. This highly

selective invasion is specific to the particular parasite and often to the particular environment. For instance, the plasmodium invades erythrocytes in which K is high and Na low; *Babesia canis* does the opposite (75).

The site of parasitic development also depends on the host reaction, so that in a sense localization may be fortuitous so far as the parasite is concerned. The survival of metastatic amoebae, for instance, may depend primarily on local tissue conditions which are suitable only because of some temporary change, for example, in circulation or Eh initiated by some general response to the infection. The collection of plasmodia and babesia along the endothelium of blood vessels and their multiplication in red cells caught up in stased vessels (but not in the extravascular erythrocytes of haemorrhages) are further examples.

Parasitic development, moreover, may be considerably affected by other host factors, including, as is well seen in plasmodial infections, the nutritional status. The evidence here suggests that in deficiencies the parasite more commonly suffers than the host (2, 4, 76, 77, 78). The development of acquired resistance, indicated in most infections by an increase in gamma globulin, may also influence either favorably or unfavorably the chance of survival of a parasite in the tissues, for example, by promoting specific phagocytosis or by causing local circulatory changes leading to more suitable conditions for growth (37, 73).

In most parasitic infections some instability of the metabolism of the host is evident. This may arise partly from organ changes resulting directly from parasitic development, for instance, the effects of local cellular hyperplasia in leishmaniasis or the destruction of erythrocytes in malaria and in babesiasis. Much more important are the general physiological effects arising from local changes in organs such as the liver or the endocrine glands, particularly the adrenals, which may appear in the local absence of parasites and dominate the whole clinical picture (17). Changes in host carbohydrate metabolism occur, for instance, in infections with plasmodia, leishmania, and the African trypanosomes (2, 24, 77, 79, 80). It is generally agreed that these do not arise directly from the metabolic activity of the parasites, even when this is notable, as in the case of the trypanosomes. They appear nevertheless to be dictated to some extent by the individual parasites, which may show differences in behaviour, probably evolutionary in character, which run parallel to differences in individual metabolic pattern. For instance, the African trypanosomes, containing heavy metal catalysts, cause profound terminal changes in the host carbohydrate metabolism associated with low blood glucose and reduction in glycogen reserve, whereas cyanide sensitive trypanosomes like *Trypanosoma cruzi*, which either do not contain such catalysts or have alternative pathways, do not (81 to 84).

Indirect effects on the host tissues arise from fever, anaemia, and circulatory disturbances, such as medical shock, or changes in hepatic and renal circulation described above in the section on malaria (32, 33). Other non-

specific effects may also develop as the result of interference with the metabolism of tissue cells, whether invaded or not.

The general physiological results of parasitic invasion have, nevertheless, some features specific to individual infections, which depend partly on the metabolism of the parasite since this determines to some extent the site of infective invasion, and partly on the kind and degree of host resistance, which, as pointed out above, may influence the distribution and well-being of the parasite in the host.

It thus appears that the invasion of the host by protozoan parasites is conditioned by both the parasite and the host. Once the invasion has begun it can produce local effects on the invaded tissues and nonspecific general effects on the host as a whole. Both local and general tissue disturbances may profoundly influence the host, for instance, by disturbing the balance of hormones or electrolytes or of the circulation. A chain reaction may follow, since these changes may in themselves lead to further physiological disturbances affecting both the host and parasites (18, 85 to 89).

The reaction of the host to invasion is thus an extremely complex synthesis of effects which are in varying degree specific and nonspecific. We have no information regarding the factor or factors which may initiate these processes, nor of those, not necessarily the same, which may be required to maintain them. No active extract or metabolic product of parasitic cells or of host cells has been identified in any infection which is capable of reproducing the wide physiological effects of parasitic invasion (90, 91). It seems, however, that changes which are basically so nonspecific probably arise from some process similar to that in acute inflammation, many of the features of which are believed to derive from the activity of relatively simple and nonspecific substances (92).

LITERATURE CITED

1. Maegraith, B. G., *Rapp. V Congr. Intern. Med. Trop., Pal.*, **1**, 50 (Imprimerie Celikcilt, Istanbul, Turkey, 1953)
2. Lwoff, A., *Biochemistry and Physiology of Protozoa*, **I** (Academic Press, Inc., New York, N. Y., 434 pp., 1951)
3. Maegraith, B. G., Deegan, T., and Jones, E. S., *Brit. Med. J.*, **II**, 1382 (1952)
4. McKee, R. W., *Parasitic Infections in Man*, 114 (Most, H., Ed., Columbia University Press, New York, N. Y., 229 pp., 1951)
5. Trager, W., *J. Parasitol.*, **33**, 345 (1947)
6. Brackett, S., Waletzky, E., and Baker, M., *J. Parasitol.*, **32**, 453 (1946)
7. Hegner, R. W., and MacDougall, M. S., *Am. J. Hyg.*, **6**, 602 (1926)
8. Maegraith, B. G., *Pathological Processes in Malaria and Blackwater Fever*, 75 (Blackwell Scientific Publications, Oxford, England, 430 pp., 1948)
9. Knisely, M. H., Stratman-Thomas, W. K., and Eliot, T. S., *Anat. Record*, **79**, Suppl., 90 (1941)
10. Rostorfer, H. H., and Rigdon, R. H., *Am. J. Physiol.*, **146**, 222 (1946)
11. Jones, E. S., Maegraith, B. G., and Sculthorpe, H. H., *Ann. Trop. Med. Parasitol.*, **45**, 244 (1951)

12. Thurston, J. P., Exptl. Parasitol., **2,** 311 (1945)
13. Foy, H., and Kondi, A., *Trans. Roy. Soc. Trop. Med. Hyg.*, **37,** 1 (1943)
14. Laser, H., *Nature*, **157,** 301 (1946)
15. Trager, W., *Ann. N. Y. Acad. Sci.*, **56,** 1074 (1953)
16. Kehar, N. D., *Records Malaria Survey India*, **6,** 499 (1936)
17. Overman, R. R., *Physiol. Rev.*, **31,** 285 (1951)
18. Overman, R. R., Hill, T. S., and Wong, Y. T., *J. Natl. Malaria Soc.*, **8,** 14 (1947)
19. Feldman, H. A., and Murphy, F. D., *J. Clin. Invest.*, **24,** 780 (1945)
20. Maegraith, B. G., *Pathological Processes in Malaria and Blackwater Fever*, 362 (Blackwell Scientific Publications, Oxford, England, 430 pp., 1948)
21. Fairley, N. H., *Proc. Roy. Soc. Med.*, **32,** 1278 (1939)
22. Rigdon, R. H., *Am. J. Clin. Pathol.*, **15,** 489 (1945)
23. Maegraith, B. G., *Brit. Med. Bull.*, **8,** 28 (1951)
24. Maegraith, B. G., *Pathological Processes in Malaria and Blackwater Fever*, 103 (Blackwell Scientific Publications, Oxford, England, 430 pp., 1948)
25. Fulton, J. D., *Ann. Trop. Med. Parasitol.*, **33,** 217 (1939)
26. Marvin, H. N., and Rigdon, R. H., *Am. J. Hyg.*, **42,** 174 (1945)
27. Dole, V. P., and Emerson, K., *J. Clin. Invest.*, **24,** 644 (1945)
28. Maegraith, B. G., *Pathological Processes in Malaria and Blackwater Fever*, 345 (Blackwell Scientific Publications, Oxford, England, 430 pp., 1948)
29. Maegraith, B. G., Jones, E. S., and Andrews, W. H. H., *Trans. Roy. Soc. Trop. Med. Hyg.*, **45,** 15 (1951)
30. Rigdon, R. H., *Am. J. Hyg.*, **36,** 269 (1942)
31. Moon, V. H., *Shock and Related Capillary Phenomena* (Oxford University Press, London, England, 442 pp., 1938)
32. Maegraith, B. G., *Trans. Roy. Soc. Trop. Med. Hyg.*, **38,** 1 (1944)
33. Trueta, J., Barclay A. E., Franklin, K. J., Daniel, P. M., and Prichard, M. M. L., *Studies of the Renal Circulation* (Blackwell Scientific Publications, Oxford, England, 187 pp., 1947)
34. Maegraith, B. G., Andrews, W. H. H., and Gall, D., *Lancet*, **II,** 781 (1947)
35. Maegraith, B. G., and Andrews, W. H. H., *Trans. Roy. Soc. Trop. Med. Hyg.*, **41,** 687 (1948)
36. Maegraith, B. G., *Pathological Processes in Malaria and Blackwater Fever*, 260 (Blackwell Scientific Publications, Oxford, England, 430 pp., 1948)
37. Taliaferro, W. H., *Malariology*, II, 935 (W. B. Saunders Co., Philadelphia, Pa., and London, England, 855 pp., 1949)
38. Coggeshall, L. T., *Medicine*, **22,** 87 (1943)
39. Maegraith, B. G., *Pathological Processes in Malaria and Blackwater Fever* (Blackwell Scientific Publications, Oxford, England, 1948)
40. Anderson, W. A. D., and Morrison, D. B., *Arch. Pathol. Lab. Med.*, **33,** 677 (1942)
41. Sadun, E. H., Carrera, G. M., Krupp, I. M., and Allain, D. S., *Proc. Soc. Exptl. Biol. Med.*, **73,** 362 (1950)
42. Hegner, R., Johnson, C. M., and Stabler, R. M., *Am. J. Hyg.*, **15,** 394 (1932)
43. Chang, S. L., *J. Infectious Diseases*, **76,** 126 (1945)
44. Deschiens, R., *Ann. inst. Pasteur*, **61,** 5 (1938)
45. Frye, W. W., and Meleney, H. E., *Am. J. Hyg.*, **24,** 414 (1936)
46. Chang, S. L., *Proc. 4th Intern. Congr. Trop. Med. and Malaria*, **2,** 1068 (1948)
47. Taylor, D. J., Greenberg, J., and Josephson, E. S., *Am. J. Trop. Med. Hyg.*, **1,** 559 (1952)

48. Kessel, J. F., *Proc. Soc. Exptl. Biol. Med.*, **27,** 113 (1929)
49. Faust, E. C., Scott, L. C., and Swartzwelder, J. C., *Proc. Soc. Exptl. Biol. Med.*, **32,** 540 (1934)
50. Anderson, H. H., Bostick, W. L., and Johnstone, H. G., *Amoebiasis*, 70 (Charles C Thomas, Springfield, Ill., 431 pp., 1953)
51. Craig, C. F., *Am. J. Trop. Med.*, **7,** 225 (1927)
52. Maegraith, B. G., Findlay, G. M., and Martin, N. H., *Nature*, **151,** 252 (1943)
53. Morita, Y., *J. Orient. Med.*, **28,** 37 (1938)
54. Harinasuta, C. (Personal communication, 1953)
55. Craig, C. F., *Etiology, Diagnosis and Treatment of Amoebiasis* (Williams & Wilkins Co., Baltimore, Md., 1944)
56. Taylor, D. J., Greenberg, J., Highman, B., and Coatney, G. R., *Am. J. Trop. Med. Hyg.*, **30,** 817 (1950)
57. Sadun, E. H., Bradin, J. L., and Faust, E. C. *Am. J. Trop. Med. Hyg.*, **31,** 426 (1951)
58. Bradin, J. L., *Exptl. Parasitol.*, **2,** 230 (1953)
59. Craig, C. F., *J. Lab. Clin. Med.*, **18,** 873 (1933)
60. Spector, B. K., *J. Preventive Med.*, **6,** 117 (1932)
61. Swartzwelder, J. C., and Avant, W. H., *Am. J. Trop. Med. Hyg.*, **1,** 567 (1952)
62. Payne, A. M. M., *Lancet*, **I,** 206 (1945)
63. Himsworth, H. P., *Lectures on the Liver and its Diseases*, 26 (Blackwell Scientific Publications, Oxford, England, 1947)
64. Maegraith, B. G., Andrews, W. H. Horner, and Wenyon, C. E. M., *Ann. Trop. Med. Parasitol.*, **43,** 225 (1949)
65. Councilman, W. T., and Lafleur, H. A., *Johns Hopkins Hosp. Repts.*, **11,** 395 (1891)
66. Carrera, G. M., and Sadun, E. H., *Am. J. Trop. Med. Hyg.*, **1,** 962 (1952)
67. Cleveland, L. R., and Sanders, E. P., *Science*, **72,** 567 (1930)
68. Kondo, H., *J. Orient. Med.*, **31,** 2, 3 (1930)
69. Reinertson, J. W., and Thompson, P. E., *Proc. Soc. Exptl. Biol. Med.*, **76,** 518 (1951)
70. Rao, V. G., *Trans. Roy. Soc. Trop. Med. Hyg.*, **45,** 3 (1951)
71. Yoshimura, B., *Keio J. Med.*, **1,** 313 (1952)
72. Carrera, G. M., and Faust, E. C., *Am. J. Trop. Med. Hyg.*, **29,** 647 (1949)
73. Maegraith, B. G., and Harinasuta, C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47,** 582 (1953)
74. Stewart, G. T., *Trans. Roy. Soc. Trop. Med. Hyg.*, **41,** 75 (1947)
75. Gilles, H. M., *Canine Babesiasis* (Bachelor thesis, Oxford University, Oxford, England, 1951)
76. Moulder, J. W., *J. Parasitol.*, **36,** 193 (1950)
77. Napier, L. E., *British Encyclopaedia of Medical Practice*, 2nd ed., **7,** 295 (Butterworth & Co., Ltd., London, England, 1951)
78. von Brand, T., Tobie, E. J., and Mehlman, B., *Am. J. Hyg.*, **54,** 76 (1951)
79. Adams, A. R. D., and Maegraith, B. G., *Clinical Tropical Diseases* (Blackwell Scientific Publications, Oxford, England, 508 pp., 1953)
80. von Brand, T., *J. Parasitol.*, **36,** 178 (1950)
81. Fulton, J. D., and Joyner, L. P., *Trans. Roy. Soc. Trop. Med. Hyg.*, **43,** 273 (1949)
82. von Brand, T., *Biochem. and Physiol. Protozoa*, **1,** 200 (Lwoff, A., Ed., Academic Press, Inc., New York, N. Y., 434 pp., 1951)

83. von Brand, T., Tobie, E. J., Kissling, R. E., and Adams, G., *J. Infectious Diseases*, **85,** 5 (1949)
84. von Brand, T., *Exptl. Parasitol.*, **1,** 60 (1951)
85. Bell, F. R., and Jones, E. R., *Ann. Trop. Med. Parasitol.*, **40,** 199 (1946)
86. Chakravarty, N. K., Sen Gupta, P. C., Bose, J. P., and De, U. N., *Indian J. Med. Research*, **37,** 113, 401 (1949)
87. Zschacke, J., *Z. Immunitätsforsch.* **107,** 90 (1950)
88. Monuet, A., Baylet, R., and Reynaud, R. *Méd. trop.*, **12,** 307 (1952)
89. Thatcher, J. S., and Radike, A. W., *Am. J. Physiol.*, **151,** 138 (1947)
90. Zwemer, R. L., and Culbertson, J. T., *Am. J. Hyg.*, **29,** C. 7 (1939)
91. Krijgsman, B. J., *Z. vergleich. Physiol.*, **23,** 663 (1936)
92. Chain, E., and Duthie, E. S., *Brit. J. Exptl. Pathol.*, **20,** 417 (1939)

HEPATITIS, YELLOW FEVER, AND DENGUE[1,2]

By W. Paul Havens, Jr.

Department of Medicine, The Jefferson Medical College of Philadelphia, Philadelphia, Pennsylvania

VIRAL HEPATITIS

The subject of viral hepatitis was reviewed in 1948 (1, 2) and again in 1953 (3). It is the purpose of this paper to discuss the more recently available information in relation to the important problems which remain unsolved. Unfortunately, serious impediments to the investigation of viral hepatitis persist, i.e., the lack of a susceptible laboratory animal as well as of a specific serologic test. However, sufficient knowledge has accumulated from experiments with volunteers and clinical and epidemiologic observations to warrant the belief that at least two forms of viral hepatitis exist. Whether these represent different diseases or variant forms of a single disease is as yet undetermined.

Terminology.—The appellative dilemma into which the nomenclature of viral hepatitis had fallen was considered in 1953 in the Report of the Expert Committee on Hepatitis, World Health Organization (4). In view of the confusion arising from the numerous names attached to the two forms of hepatitis now recognized, it was recommended, in accordance with MacCallum's (5) original suggestion, that the epidemic or sporadic form of the naturally occurring disease be called "infectious hepatitis" and that the agent causing it be termed hepatitis virus A. Although no strict recommendation was made concerning the name to be applied to the artificially transmitted disease, the term "serum hepatitis" was used throughout the report, and it was recommended that the agent causing it be called hepatitis virus B. The inadequacy of the term serum hepatitis is recognized, since virus B may be found not only in serum but in whole blood and some of its derivatives. In addition, the arbitrary definition of a disease on the basis of route of inoculation of its causative agent may be confusing, since virus A may also be found in the blood and transmitted parenterally.

Infectious Hepatitis

Hepatitis virus A.—Many of the numerous attempts to transmit hepatitis virus A to a variety of experimental animals have been summarized by Colbert (6). They have been either unsuccessful or, in those instances where transmission was reported to have been accomplished, the results have not been confirmed. Further examples of the spontaneous appearance of filtrable

[1] The survey of literature pertaining to this review was completed in March, 1954.

[2] Sponsored by the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, and supported in part by the Office of the Surgeon General, Department of the Army.

(7 to 11) and nonfiltrable (12) agents causing hepatitis in mice have been described, but there is no recognized relationship between such agents and those producing hepatitis in man. Transmissible forms of hepatitis were described in birds (13), mice (12, 14 to 17), and rats (18) following inoculation with potentially infectious materials obtained from patients with hepatitis. There is no evidence, as yet, to indicate that the disease which appeared in any of these species is related to human hepatitis. The results of attempts to propagate hepatitis virus A on the developing chick embryo or in cultures of various human and animal tissues (6) have been inconsistent. Although several workers have described the growth of virus A in such media, no characteristic lesions or identifying tests have been found, and the only means of demonstrating virus in such materials is by inoculation into man. Henle and associates (19) reported the growth of virus A in tissue culture and embryonated eggs, and the virus was re-identified by suggestive but not conclusive tests in volunteers (20). These workers have also used (21, 22, 23) amniotic fluid containing presumably inactivated hepatitis virus A for making skin tests, and have found a high percentage of positive tests in patients recovered from infectious hepatitis. An apparent reduction in incidence of hepatitis in one institution among children who had received the skin test material raised the question as to whether this might indicate an immunizing effect (24). Unfortunately, the technical difficulties involved have made it impossible to obtain consistently reproducible results, and all of these observations await confirmation by others.

Since no satisfactory experimental animal is known to be susceptible to virus A, our knowledge of this agent is dependent on the results of experiments in volunteers. Because of the natural limitations of this method, negative results cannot be regarded as of statistical significance. Little is known about the size and properties of this virus, although it passes a Seitz EK filter and is apparently highly resistant to a number of physical and chemical agents. It is found in the blood, duodenal contents, and feces of patients in the acute phase of disease, and may be transmitted experimentally by oral and parenteral inoculation. Homologous immunity exists following infections caused by virus A, but there is no heterologous immunity against infections caused by virus B (1).

The duration of infectivity is not known exactly. Earlier limited studies (1) indicated that virus was no longer present in the blood or feces one and three months after onset; however, more recent studies by Capps and his associates (25 to 28) demonstrated the presence of virus in the feces of two children with persistent evidence of hepatitis five and sixteen months, respectively, after the onset of disease.

Epidemiology.—The importance of infectious hepatitis as a problem of world health has been emphasized (29) by its continued prevalence in both civilian and military populations during the past twelve years. The disease is still not reportable to public health authorities in most places so that an idea of its incidence in many areas can be gained only from descriptions of

sizable outbreaks. Attention has been called to the long-term variation in prevalence, with epidemics and pandemics at irregular intervals (30). During the past few years, civilian and military outbreaks have furnished observations corroborating and extending previously available information. In addition, certain new information has become available on the "carrier state" and its possible role in transmission of disease.

Interesting descriptions of the pattern of hepatitis among families (31), inmates of institutions (25 to 28, 32, 33, 34), residents of housing projects (35), and other civilian groups (30, 36) have appeared. In most instances, direct contact has been believed to be the way of spread. However, at least two outbreaks presumably initiated by contaminated food (37) and water (38) have been described. Although epidemics involving goodly numbers of adults (39) have occurred, the usual predilection of the disease for younger persons, particularly the age group between five and fourteen years, has been observed. Of particular interest were the epidemiologic data made available by the studies of two groups working in orphanages in England (30) and the United States (25 to 28). Attention was initially called to the high incidence of disease in nurses and the apparently low incidence among the children. However, further investigation revealed (25 to 28) that hepatitis without jaundice was widely prevalent among the small children, and it was suggested that these children served as a source of infection to the nurses.

Lilienfeld *et al.* (35) described an outbreak of hepatitis in a housing project where the over-all attack rate was somewhat higher for females (3.4 per cent) than males (2.3 per cent) but where the annual attack rate among older girls of fifteen to nineteen was much greater (4 per cent) than that occurring in boys (0.6 per cent). This observation in conjunction with the increased mortality rates among women during pregnancy or after the menopause suggests the possibility that certain specific factors related to sex may affect the course of disease.

Previously observed differences in secondary attack rates between urban and rural areas (40) were also found in a recent urban outbreak (35) in the United States where the secondary attack rate measured 8.8 per cent; this was considerably lower than the rates of 20 to 40 per cent usually reported among civilians in rural areas in the United States. The risk of infection in various households involved in this outbreak as well as others (30) apparently depended on the degree of proximity to an already infected household; marked increase of the risk was found only in closely-situated households. This was interpreted to support the concept that the fecal-oral route is the most important way of spread since such strict geographic limitations would probably not characterize an air-borne spread of disease.

Of great interest is the role of carriers in the spread of hepatitis. The duration of infectivity is not known exactly but the evidence suggests that there is, in most cases, a relatively short period of infectivity in the acute phase of disease, although some cases may be infectious earlier or later. Epidemiologic evidence suggests that carriers play an important role in the spread of dis-

ease. In this regard, MacCallum *et al.* (30) called attention to the following evidence: (*a*) the frequent lack of any history of contact of patients with known cases of hepatitis; (*b*) the occurrence of cases in one village frequently following contact in another village where the disease had occurred but was apparently no longer active; (*c*) the immunity in older life, doubtlessly attributable to subclinical infection in younger life. In support of the concept that carriers may be important in the maintenance of hepatitis are the important observations of Capps and his associates (25 to 28) mentioned above, in which virus was recovered from the feces of two children five and sixteen months, respectively, after the onset of hepatitis.

The high incidence of disease among U. S. Occupation troops in Germany, with rates ranging from six to eighteen per thousand per annum from 1945 to the present (41 to 44) has been an important problem. The usually observed seasonal variation was less evident, although somewhat higher rates prevailed during the winter months. Localized outbreaks were uncommon and the disease was spread diffusely throughout the Command. The explanation of this endemiologic pattern is unknown. The American Zone, made up largely of Bavaria, has long been recognized as an endemic focus of hepatitis (45). The use of human feces for fertilizer was widespread, and Burlingame *et al.* (46) commented on the high incidence of intestinal parasites among troops. The possibility also that increased fraternization played a part in maintaining the high rate of infection has been mentioned. An investigation of the duration of residence in Germany before the onset of hepatitis revealed that in 1946 and 1947 it was uncommon for men to contract hepatitis before the fifth month of residence in Germany, and that most men came down with the disease from six to thirteen months after arriving (41). Studies made three years later on groups remaining in Germany for longer periods revealed that it was still uncommon to acquire hepatitis before four months' residence in the country but, although the rate rose after that to reach a peak in twelve months, it declined slowly so that it was not until after thirty months that the rate could be said to be low (43). This endemiologic situation was quite different from the experience reported by Gauld (47) in the Mediterranean campaign and by Havens (48) in Korea in 1950 and 1951 when fresh troops acquired hepatitis soon after arriving under conditions of combat and had a considerably higher incidence of disease than did seasoned troops.

Whether the initial long period of four to five months' delay before acquiring hepatitis in Germany may have been the result of delay in natural exposure or of artificial transmission of virus with a longer incubation period is unknown. Some support for the latter possibility is furnished by the work of Evans (49) who, with virus recovered from the blood of a soldier with hepatitis, produced the disease in volunteers only after a long incubation period following parenteral inoculation.

Clinical course of disease.—Little new has been added by the descriptions of the clinical course of hepatitis which have appeared during the past five years (50 to 55). Leibowitz (56) called attention to distaste for smoking as

an early symptom, and the rarity of neurologic or neuropsychiatric complications except in those severely sick or in fatal cases was described (57, 58). The frequency of the disease among children (59 to 64) and increased emphasis on the importance of nonicteric cases (65 to 68), particularly in children but also in adults, were the subjects of several reports.

Although wide variations in the course of disease have been described, the majority of accounts have dealt with rather mild disease, with a mortality rate similar to that previously reported ($<2:1000$). Occasional exceptions to this have been described, more particularly among females, although men have also been involved in outbreaks of severe disease (69, 70). Of particular interest during the past few years was the clinical experience in Germany with United States Occupation troops (71). In these patients, the course of disease was mild and not unusual with the exception of the type of onset which was insidious in a large proportion of patients; fever was uncommon. The explanation of these differences is not known, but the question has been raised as to whether a certain proportion of these patients did not have artificially transmitted disease which characteristically has this type of onset. Some support for this concept was provided by Evans (49) who produced hepatitis with jaundice in three volunteers fifty-six to eighty-seven days after inoculation of plasma obtained from a soldier with hepatitis in Germany.

Chronic hepatitis.—In spite of the fact that reports continue to appear describing the failure of certain patients with hepatitis to make a complete or sustained recovery (72, 73, 74), it is evident that significant functional impairment is uncommon (75) and that only a small percentage of patients develop severe chronic hepatic disease.

Attention has also been called to the lack of correlation between the incidence of subjective and objective findings in patients who have ostensibly recovered from hepatitis (76, 77). The most common and reliable manifestations of hepatic damage among such patients are chronic or recurrent hyperbilirubinemia and impaired capacity to excrete bromsulfalein. In most of such cases, the degree of functional impairment of the liver and the clinical condition are apparently compatible with full activity and eventual recovery.

Laboratory studies.—Reports of the occurrence of a number of nonspecific and apparently different serologic reactions in patients with viral hepatitis have been reviewed (78), and attention was called to the variable frequency of their occurrence, according to the results of different workers. Other efforts to develop a specific serologic reaction revealed that the serums of a large percentage of patients with hepatitis have the capacity to fix complement in the presence of antigens made from extracts of the feces of both normal persons and patients with hepatitis (79). Although the exact nature of this reaction is not known, certain characteristics suggest that it was heterophile in type. Evans (80) described the agglutination of human erythrocytes modified by Newcastle Disease virus by serums of patients in the

convalescent phase of the disease. The development of heterophile antibody in a titer of >1:56 in 20 per cent of convalescent patients was recorded by Leibowitz (81). With the exception of one earlier report, this is in contrast to the results of others (78). Using gamma globulin prepared from the serums of patients convalescent from hepatitis as a source of antibody, Havens & Eichman (78) attempted to detect virus in the serums of patients in the acute phase of disease. Collodion particles, sensitized with acute phase serums from patients with hepatitis as well as certain other infectious diseases, were agglutinated when they were incubated with convalescent hepatitis gamma globulin. The nature of the reagin in the acute phase serums was not clear, although the available evidence suggested it represented a nonspecific alteration of lipoproteins in acute phase serums. Recently, Pollard & Bussell (82) reported again the presence of an antigen in the acute phase serum of a patient with serum hepatitis which fixed complement with serums from other patients with serum hepatitis but not with serums from persons with infectious hepatitis. This is reminiscent of earlier reports (78); however, with evidence available at present, there is no reason to assign any specificity to this reaction.

Numerous reports on a variety of biochemical alterations occurring during hepatitis which are not of primary interest to this review have appeared. For the purpose of reference, the following subjects have been discussed: serum proteins (83 to 87); cephalin-cholesterol flocculation and thymol turbidity tests (88 to 96); serum mucoproteins (97) and C-reactive protein (98, 99); serum lipids (100, 101); serum cholinesterase (102 to 106); serum iron (107, 108); urinary coproporphyrin (109, 110); excretion of bromsulfalein (111); urinary bilirubin and urobilinogen (112); urinary amino acids (113, 114); urinary steroids (115).

Treatment.—Swift *et al.* (71) attempted to determine whether the duration of rest in bed might be shortened, and they showed that previously healthy young men had no obvious prolongation of disease if vigorous exercise was begun when the total serum bilirubin measured 3 mg. per cent or less or if the retention of bromsulfalein in the blood measured 10 per cent or less. Follow-up studies of three large groups of men for six to twelve months (71, 77) and for two to three years (116) after recovery revealed no evidence of residual hepatic damage. A recent carefully controlled study from the Hepatitis Center of the United States Armed Forces in Japan revealed that allowing men out of bed for most of the time from the first day of hospitalization for hepatitis did not prolong the course of disease (117).

Reports of the use of vitamin B_{12} (118), various amino acids (119), brewer's yeast (120), choline (121), and methionine (122) have appeared, but little evidence was furnished to indicate that any of these substances added anything to the treatment of the disease. Chalmers *et al.* (117) in a well-controlled study found that the course of disease was shortened by one week in patients who ingested a diet containing from 3000 to 4000 calories, providing protein was present in amount of 19 per cent.

Numerous reports on the use of broad-spectrum antibiotics have appeared (123 to 129), but their effectiveness, except possibly in patients chronically sick or in hepatic coma (124, 130 to 134), has not been demonstrated. The mechanism of favorable action in such patients is undetermined, although it has been suggested that alteration of the gastrointestinal flora may change the metabolic load of the liver (135, 136). On the other hand, it has also been shown that the administration of aureomycin apparently was associated with increased amounts of fat in the liver which may be accompanied by functional abnormalities (137).

It has been shown that the administration of adrenocorticotropin (ACTH) or cortisone to patients in the acute phase of viral hepatitis produces a sense of well-being, improvement in appetite, a sharp diminution in serum bilirubin, and a slight shortening of course of disease (138 to 144). However, the occurrence of other effects, including ascites, edema, "mooning" of the face, and hypertension, and the frequency of relapse following cessation of too short a course of therapy were so common as to preclude the use of these materials in the average case. Nevertheless, in patients with fulminant severe hepatitis, these substances in combination with broad-spectrum antibiotics have been reported to be of therapeutic value (145, 146). Further investigation of this therapy is required.

Prevention.—Although the period of infectivity is not well defined, it has been recommended that the feces be regarded as infectious for three weeks after onset. In addition, the necessity of adequate sterilization of any instruments coming in contact with the blood of patients has been emphasized (4). Specifically, normal human gamma globulin prevents the disease, and as little as 0.01 ml./lb. of body weight, administered intramuscularly, is usually effective (147). This type of prophylaxis is ordinarily reserved for household contacts.

The duration of passive immunity after receiving gamma globulin has been estimated to range from six to eight weeks, although observation of groups of children inoculated with gamma globulin and subsequently heavily exposed to hepatitis virus for a prolonged period suggested that a more permanent form of protection may occur. Stokes and associates (36) have suggested that active immunity may have been superimposed on passive protection under such conditions.

Serum Hepatitis

The importance of serum hepatitis has increased with the widespread use of whole blood and certain of its derivatives and the lack of reliable methods of sterilizing most of these materials of hepatitis virus. Observations on the incidence of serum hepatitis following the use of whole blood or plasma (147 to 153) have shown the incidence of disease to range from 0.2 to 0.8 per cent for whole blood, from 0.96 to 1.3 per cent for plasma from small pools, and up to as high as 11.9 per cent for plasma from large pools. More recent observations by Sborov *et al.* (154) on the incidence of disease re-

vealed that 21.9 per cent of wounded American troops in Korea receiving blood and plasma (both irradiated and nonirradiated) contracted hepatitis, while 3.6 per cent who received whole blood alone contracted the disease. Its occurrence in patients treated with dried serum (155) and in patients given therapeutic malaria (156, 157) has been described, and other reports have appeared concerning numerous other potential sources of infection. Fibrinogen (158), thrombin[3] (159, 160), and Cohn's Fraction IV (161) may be vehicles for the transmission of virus B, and the probability is good that packed red cells are potentially infectious.

The accumulated data suggest that human albumin and gamma globulin have little or no role in the transmission of serum hepatitis. Clinical studies of recipients of albumin[4] (162) have corroborated earlier and subsequent experimental observations (163) that albumin is not infectious. Likewise, although two cases of hepatitis following the use of gamma globulin have been reported (164, 165), it is evident that the risk is negligible in view of the tremendous number of injections of this material which have been given (166, 167, 168). Of particular interest in this regard is the study of Cockburn *et al.* (165) who reported that seven out of ten children who received convalescent measles plasma from a single pool contracted serum hepatitis, while only 1 out of 58 children who received the gamma globulin fraction of this same pool contracted hepatitis.

The importance of contaminated instruments, particularly syringes and needles, has been re-emphasized (169, 170, 171), and additional reports of the occurrence of hepatitis among patients receiving vaccinations (172), therapy for syphilis (173, 174, 175), tuberculosis (176), and pernicious anemia (177), and in diabetics (178) have appeared. The potential danger of transmission by dental manipulations has been suggested (42, 179, 180), and the transmission by tatooing has been described (181 to 183). Attention has also been called to the occurrence of artificially transmitted hepatitis among narcotic addicts and the possibility of their being an important source of carriers (184 to 187). The disease is recognized as an occupational hazard among physicians (188) and various other medical personnel (189, 190, 191), and precedent has been set for it to be compensable (192).

Hepatitis virus B.—The results of numerous unsuccessful experiments to transmit hepatitis virus B to animals, developing chick embryos, and tissue culture have been reviewed by Colbert (6). Up to the present time, the only means of demonstrating virus is by inoculation into man. Henle *et al.* (193) described experiments indicating the possibility of growth of virus B in tissue culture. However, the results of their attempts to reidentify the virus by inoculation into volunteers were only suggestive and not conclusive.

The available information about hepatitis virus B has been derived

[3] Thromboplastin (placental extract) used as activator might have contained the virus.

[4] Heating to 60° C. for 10 hr. is a part of the commercial preparation of human albumin.

largely from experiments in its transmission to volunteers. Virus B is highly resistant to various physical and chemical agents. It is found in the blood during the long incubation period and in the acute phase of disease, and it may be transmitted experimentally by parenteral inoculation. It has not been found in the feces. The status of homologous immunity in infections caused by virus B is equivocal, and heterologous immunity does not exist between the disease caused by this agent and that caused by virus A. Normal human gamma globulin apparently rarely protects against serum hepatitis.

The exact size of virus B is not known. Earlier observations indicated that it passed a Seitz EK filter, but McCollum (194) showed that it may also pass through a membrane of 52 mμ average pore diameter, indicating a size of 26 mμ or less.

The duration of infectivity of patients with serum hepatitis is not known. Limited earlier experiments indicated that virus was no longer present in the blood one month after the onset of disease, although more recent observations have indicated (195, 196, 197) that certain individuals with or without evidence of hepatic disease may remain carriers of virus for as long as five years.

Epidemiology.—Serum hepatitis occurs among all age groups, although it has been suggested that children may be more resistant to it than adults (167) and that the disease in them is less severe. However, this need not always be true since serious outbreaks among infants, with high mortality, have been recorded (165). Young adults are highly susceptible as shown by the experience of recent years, and when the disease is contracted by otherwise healthy persons, as in troops inoculated with yellow fever vaccine, the severity of disease and the mortality are similar to those found in epidemic disease. However, when patients of any age are previously debilitated by illness or wounds, the mortality rates increase rapidly (198, 199), and particularly among older women the disease has been severe.

The apparent failure of normal human gamma globulin to prevent serum hepatitis consistently in field studies has been reinvestigated by Stokes and associates (200, 201), using volunteers who were inoculated with virus B and gamma globulin prepared from persons convalescent three months to three years from serum hepatitis. The fact that no protection was observed in these experiments reemphasizes the lack of knowledge concerning the immune response following infection with virus B.

Prevention.—The high degree of resistance of virus B to various physical and chemical agents and the necessity of using volunteers to determine infectivity have limited knowledge on the exact amounts of various agents required to kill it. Earlier observations indicated that it survived (in serum) a temperature of 60°C. for one hour but was destroyed (in human albumin) by heating to 60°C. for ten hours. However, Murray & Diefenbach (163) showed that this virus may survive (in albumin) a temperature of 60°C. for as long as four hours.

Numerous attempts to find methods of sterilizing whole blood or plasma

of virus B have been made. Preliminary observations (202, 203) suggested that irradiation of plasma with ultraviolent light destroyed the virus, but subsequent studies have indicated that experimentally this is not reliable, and multiple cases of hepatitis have been reported to follow the injection of irradiated plasma (204 to 211). It is not determined whether such occurrences represent intermittent breakdowns in the apparatus for irradiation, alterations in strain or amount of virus, or the condition of the plasma which may determine the absorption of irradiation. However, it may be said at the present time that the intensity and conditions of ultraviolet radiation necessary to rid plasma of viable hepatitis virus are not known, and that this method of sterilization is not adequate.

Investigations of the possibility of chemical sterilization of plasma and blood (212) with nitrogen mustards have been made, but Drake *et al.* (213) showed that hepatitis virus B survived in blood containing nitrogen mustard in concentration of 500 mg./l. Further investigations of other methods of chemical sterilization are in process.

Recent clinical studies by Allen *et al.* (214, 215) of recipients of plasma which had been stored in the fluid state at room temperature for from three to six months indicated that the incidence of serum hepatitis was so low as to suggest that inactivation of virus might have occurred. Experimental data obtained from volunteers by Murray *et al.* (216), however, indicate that virus B may survive storage at room temperature in plasma for from three to six months.

Because of the small size of the virus and its relative resistance to heat and other physical and chemical agents, it has been recommended (4) that every parenteral penetration be performed with instruments sterilized by boiling in water for at least 10 min., or with steam under increased pressure (15 to 20 lbs.), or with dry heat (180°C. for 1 hr.). The importance of thorough cleansing of all instruments before attempts at sterilization has been emphasized. Because of the carrier rate (as much as 0.5 per cent in one survey of donors), the necessity for carefully evaluating the need of transfusion of whole blood or plasma in each patient is evident. In addition, the statistical advantage of utilizing plasma made from small groups (10 to 20) of donors is apparent. It has been further recommended (4) that, when circumstances permit, no donor should be accepted if he has a history of hepatitis except under conditions of dire emergency for single transfusions, or under conditions in which his blood may be used in the preparation of a product such as albumin which can apparently be sterilized of hepatitis virus.

YELLOW FEVER

It is the purpose of this section to discuss briefly certain publications concerned with the epidemiology and control of yellow fever which have appeared since the authoritative description in 1951 by Strode *et al.* (217) of research and development in our knowledge of this disease.

Epidemiology.—The endemic zones of yellow fever in Africa and America have been delineated in two maps by the World Health Organization (218), and a number of epidemiologic descriptions of the disease in Bolivia (219), Colombia (220), Ecuador (221, 222, 223), Panama (224), the Caribbean area (225, 226), Argentina (227), and Brazil (228) have appeared. Bevier *et al.* (219) called attention to the pattern of widely distributed cases in 1949 and 1950 occurring in areas previously free of disease in Bolivia. *Haemagogus* mosquitoes which had left their natural habitat after an unusually wet season and become house pests were thought to be the vectors. Komp (229) observed that although the natural breeding places of *Haemagogus spegazzinii falco* are tree holes these mosquitoes may breed at ground level in areas where human blood is available. Gast Galvis (220) described cycles of prevalence of sylvatic yellow fever in Colombia every four to six years, and Kumm (230) also called attention to this periodicity in South America and Africa.

Of particular importance were outbreaks of yellow fever in Panama and Costa Rica (224, 225, 231) and the descriptions of Elton (232, 233, 234) of sylvan yellow fever spreading west to the coast and then north at a rate of twelve to fifteen miles per month in Costa Rica and Nicaragua. Transmission to man is believed to be effected by *Haemagogus spegazzinii falco* and *Aëdes leucocelaenus*, although *Sabethes chloropterus* (235, 236) is also under suspicion. The epizootic was reported to have spread from Central America along the coastal plains of Ecuador, and Levi-Castillo (221, 222) reported a fresh outbreak of 53 cases of human disease in 1951.

Animal reservoirs.—It has been emphasized that in Uganda yellow fever is essentially a disease of monkeys, transmitted among monkeys by *Aëdes africanus* and to man by *A. simpsoni* (237), although a subsidiary route of infection may be *A. africanus* carrying virus to plantation workers (238). A serologic survey (239) stimulated search for undiscovered reservoirs, and Dick (240) found hedgehogs highly susceptible and genets moderately so. A study of nonprimates by de Rodaniche (241) in Panama revealed 2 of 13 agoutis and 1 of 5 pigmy anteaters to have positive neutralization tests. Unfortunately, the results of these tests were not always consistent and are difficult to interpret.

Vaccine.—In Bwamba County 77 per cent of vaccinated persons and 17.5 per cent of nonvaccinated persons were immune for nine years after mass subcutaneous vaccination with the 17D strain. Because of these findings, Dick & Gee (242) suggested that the validity of yellow fever certificates could be extended to at least nine years after vaccination except in children who were under seven years of age when vaccinated.

In spite of the high incidence of postinoculation reactions, Mathis (243) claimed that the French vaccine prepared from mouse brain was superior to the vaccine made with the 17D strain in chick embryos. In an epidemic in Southern Nigeria, Macnamara (244) described numerous mild reactions with occasional evidence of viscerotropic reactions in persons who were vaccinated

by scarification with a French neurotropic strain. Of particular importance, however, was the experience with neurotropic disease following the use of this material in Eastern Nigeria where encephalitis occurred in from 3 to 4 per 1000 vaccinations among children, with a case fatality of 40 per cent. Adults were less frequently affected. The strain of yellow fever virus used in preparation of the vaccine was recovered from three of four brains examined (245).

Hahn (246) developed a combined vaccine to be administered by scarification, using 17D virus and vaccinia virus. The immunity to 17D developed more slowly, but tests carried out several months after vaccination revealed immunity in from 92 to 95.6 per cent. Dick (247) showed that although scarification with 17D gives good antibody it is not as effective as subcutaneous inoculation of 17D or scarification with the French neurotropic strain. Dick & Horgan (248) compared the development of neutralizing antibody in two groups of females, one of which was inoculated by scarification with a combined vaccine of 17D and vaccinia and the other with 17D given subcutaneously and vaccinia by scarification. Neutralizing antibody to yellow fever virus appeared in 14 out of 21 persons in the first group and in all of the 22 of the second group. The reaction to vaccinia was the same in both groups.

Hahn & Bugher (249) defined the stability of various lots of chick embryo yellow fever vaccine stored in a desiccated state for from five to seven years at temperatures of 0 to 4°C. At the end of one year, titers had declined 1.5 to 2.0 logarithmic units, and only a slight decline in potency occurred in the following five years.

DENGUE

During the past decade, a great deal of new knowledge of the physical and biologic properties of the dengue viruses has been made available. Many of the investigations were carried out in volunteers, and the major part of this important work was done by Sabin and his associates during and after World War II. Excellent reviews of it were published in 1950 (250) and 1952 (251). Information about the size, infectivity, and resistance to certain physical and chemical agents of dengue viruses was gained. One strain of virus was adapted to mice, with the subsequent appearance of a variant which was successfully propagated in embryonated eggs and which produced a modified disease in volunteers followed by the development of immunity and neutralizing antibody, but not complement-fixing antibody. This mutant was also used to make an effective vaccine. A dermal neutralization test in man (250, 251), a neutralization test in mice (252), and a complement fixation test (253) were developed. At least two immunologically different groups of dengue viruses were defined, and long-lasting immunity to reinfection with homologous strains was demonstrated with short-term resistance to reinfection with heterologous strains. The association of homologous

neutralizing antibodies with type-specific immunity was demonstrated, and the use of neutralization and complement fixation tests for epidemiologic and diagnostic surveys was developed. The determination of the existence of different immunologic types of dengue with a lack of enduring heterologous immunity, and the demonstration of modifications of clinical disease resulting from reinfection with heterologous types of virus potentiated recognition of the fact that many short, febrile diseases previously not regarded as dengue were, in actuality, this disease (250, 251).

Properties of virus.—Dengue virus passes a gradocol membrane of an average pore diameter of 75 mμ, indicating a size of from 17 to 25 mμ. It may be sedimented from human serum by centrifuging at a speed of 24,000 r.p.m. for 90 min. Preparations made from infective human serum were found to contain dumbbell shaped bodies (700 mμ×20 to 40 mμ) (251). Reagan & Brueckner (254) described rodlike bodies from 42 to 46 mμ wide and from 175 to 220 mμ long in preparations made from mouse brain infected with a mouse-adapted Hawaiian strain of virus. The relationship of these particles or those previously described by Sabin (251) to dengue virus is not known. Dengue virus remains active for at least five years in human serum frozen at dry-ice box temperature or lyophilized and stored in an ordinary refrigerator (250). It is inactivated in human serum or in suspensions of mosquitoes containing 0.05 per cent formalin or by exposure to ultraviolet light (0.47 sec. in an Oppenheimer-Levinson apparatus) (250, 251).

Host range.—Unmodified human dengue viruses produce inapparent infections in rhesus monkeys and chimpanzees (255) following intracerebral or intraperitoneal inoculation, evoking within 14 to 21 days neutralizing and complement-fixing antibodies which may persist for many months (250). Several strains of virus have been adapted to young mice by serial intracerebral passage (252, 256, 257), although wide variations in pathogenicity have been observed (250, 251). Schlesinger & Frankel (258), working with "New Guinea B" strain of virus, found that serial passage in baby mice increased the pathogenicity for adult mice. They postulated that the virus appearing in early baby mouse passage consisted of two types of particles, one pathogenic for baby mice and the other for adult mice, with the predominance of the latter type developing on continued passage. Meiklejohn *et al.* (259) also emphasized that unweaned mice were generally more susceptible than older mice to infection with five different strains of virus, and the same workers described the successful transmission of two strains of mouse-adapted dengue virus to unweaned hamsters (260). Reagan & Brueckner (261) reported serial transmission of dengue virus in bats, and Schlesinger (262) extended earlier observations (250, 251) that the mouse-adapted Hawaiian strain could be cultivated serially in embryonated eggs without loss of titer, pathogenicity for mice, serologic specificity, or capacity to produce in volunteers a modified disease.

Immunity.—The seven strains of dengue virus described by Sabin (250,

251) and three strains described by Japanese workers (256) and subsequently identified by Sabin (250, 251) fall in two immunologically specific groups (251, 258). Seven strains (Hawaiian, New Guinea A, two strains from India, and three from Japan) belong to one group, and three other strains (New Guinea B, C, and D) belong to the second group. Type-specific active immunity persists in volunteers for as long as 18 months after infection, and immunity to heterologous strains may last from one to two months, probably on the basis of group-specific common antigen. Reinfection with heterologous virus from two to nine months after initial infection usually results in the appearance of a mild, short disease without rash. By injecting the skin of volunteers with a mixture of acute dengue serum and serum from patients in various periods after onset of disease, Sabin (250, 251) determined that type-specific neutralizing antibodies were present from one week to eight months after defervescence. Of interest was the fact that during the first two months after onset of disease when heterologous immunity was present, only type-specific antibody was demonstrated in the dermal neutralization test. Neutralization tests utilizing mouse-adapted virus established the fact that the neutralizing antibody was type-specific, appearing as early as one week after onset and persisting for as long as four years. Homologous complement-fixing antibody appears early after infection and may persist in man for prolonged periods. Experimentally, there is apparently a degree of serologic cross relationship between the two groups of virus (250, 251).

Relationship of dengue to other viruses.—Experiments with volunteers inoculated with 17D yellow fever virus and dengue virus at intervals as long as one week apart revealed interference between the two viruses, and similar interference was also shown in rhesus monkeys using a viscerotropic strain of yellow fever virus. Interference with multiplication of smaller doses of yellow fever virus in mosquitoes previously injected with dengue was also suggested. Antigenic interrelationship between the dengue viruses and those of yellow fever, Japanese B encephalitis, and West Nile fever was demonstrated by the use of complement fixation tests (250, 251).

LITERATURE CITED

1. Havens, W. P., Jr., *Medicine*, **27**, 279 (1948)
2. Gellis, S. S., and Janeway, C. A., *New Engl. J. Med.*, **239**, 503 (1948)
3. Gellis, S. S., and Hsia, D. Y. Y., *New Engl. J. Med.*, **249**, 400 (1953)
4. *World Health Organization Tech. Rept. Ser. No. 62* (1953)
5. MacCallum, F. O., *Trans. 4th Meeting Intern. Soc. Geographical Pathol.*, p. 274 (Liège, Belgium, 1952).
6. Colbert, J. W., Jr., *Yale J. Biol. Med.*, **21**, 335 (1949)
7. Gledhill, A. W., and Andrewes, C. H., *Brit. J. Exptl. Pathol.*, **32**, 559 (1951)
8. Gledhill, A. W., Dick, G. W. A., and Andrewes, C. H., *Lancet*, **II**, 509 (1952)
9. Steigman, A. J., *Proc. Soc. Exptl. Biol. Med.*, **80**, 177 (1952)
10. Nelson, J. B., *J. Exptl. Med.*, **96**, 293 (1952)

11. Nelson, J. B., *J. Exptl. Med.*, **96,** 303 (1952)
12. Lackey, M. D., Eichman, P. L., and Havens, W. P., Jr., *J. Infectious Diseases*, **93,** 14 (1953)
13. Lucké, B., and Radcliffe, H., *Trans. Assoc. Am. Physicians*, **62,** 83 (1949)
14. Kimura, R., and Hotta, S., *Acta Schol. Med., Univ. Kioto*, **30,** 133 (1952)
15. Hara, K., Kashiwagi, Y., Obayashi, A., Tamamura, Y., Tsuchiya, T., Sugiyama, T., Hatano, S., and Yasukochi, H., *Proc. Japan Acad.*, **28,** 270 (1952)
16. Hara, K., *et al.*, *Proc. Japan Acad.*, **28,** 511 (1952)
17. Jordan, J., and Mirick, G. S., *Bull. Johns Hopkins Hosp.*, **89,** 326 (1951)
18. Melcher, G. W., Jr., Blunt, J. W., and Ragan, C., *J. Clin. Invest.*, **31,** 649 (1952)
19. Henle, W., Harris, S., Henle, G., Harris, T. N., Drake, M. E., Mangold, F., and Stokes, J., Jr., *J. Exptl. Med.*, **92,** 271 (1950)
20. Drake, M. E., Kitts, A. W., Blanchard, M. C., Farquhar, J. D., Stokes, J., Jr., and Henle, W., *J. Exptl. Med.*, **92,** 283 (1950)
21. Stokes, J., Jr., Henle, G., Drake, M., and Henle, W., *Trans. Assoc. Am. Physicians*, **63,** 122 (1950)
22. Henle, G., Drake, M., Henle, W., and Stokes, J., Jr., *Proc. Soc. Exptl. Biol. Med.*, **73,** 603 (1950)
23. Stokes, J., Jr., *Ciba Foundation Symposium on Liver Disease*, 235 (The Blakiston Co., Philadelphia, Pa., 249 pp., 1951)
24. Drake, M. E., Ward, C., Stokes, J., Jr., Henle, W., Medairy, G. C., Mangold, F., and Henle, G., *J. Exptl. Med.*, **95,** 231 (1952)
25. Capps, R. B., Bennett, A. M., and Stokes, J., Jr., *J. Clin. Invest.*, **29,** 802 (1950)
26. Capps, R. B., Bennett, A. M., and Stokes, J., Jr., *Arch. Internal Med.*, **89,** 6 (1952)
27. Capps, R. B., and Stokes, J., Jr., *J. Am. Med. Assoc.*, **149,** 557 (1952)
28. Bennett, A. M., Capps, R. B., Drake, M. E., Ettinger, R. H., Mills, E. H., and Stokes, J., Jr., *Arch. Internal Med.*, **90,** 37 (1952)
29. McNee, J., *Brit. Med. J.*, **I,** 1367 (1952)
30. MacCallum, F. O., McFarlan, A. M., Miles, J. A. R., Pollock, M. R., and Wilson, C., *Med. Research Council (Brit.), Spec. Rept. Ser. No. 273* (1951)
31. Brooks, B. F., Hsia, D. Y. Y., and Gellis, S. S., *New Engl. J. Med.*, **249,** 58 (1953)
32. Brodribb, H. S., *Lancet*, **I,** 339 (1952)
33. Farquhar, J. D., *Philadelphia Medicine*, **45,** 335 (1949)
34. Stokes, J., Jr., *American Journal of Public Health*, **43,** 1097 (1953)
35. Lilienfeld, A. M., Bross, I. D. J., and Sartwell, P. E., *Am. J. Public Health*, **43,** 1085 (1953)
36. Stokes, J., Jr., Farquhar, J. A., Drake, M. E., Capps, R. B., Ward, C. S., Jr., and Kitts, A. W., *J. Am. Med. Assoc.*, **147,** 714 (1951)
37. Kaufmann, G. G., Sborov, V. M., and Havens, W. P., Jr., *J. Am. Med. Assoc.*, **149,** 993 (1952)
38. Farquhar, J. D., Stokes, J., Jr., and Schrack, W. D., Jr., *J. Am. Med. Assoc.*, **149,** 991 (1952)
39. Ipsen, I., Jr., *Am. J. Hygiene*, **51,** 255 (1950)
40. Ford, J. C., *Lancet*, **I,** 675 (1943)
41. Paul, J. R., and Gardner, H. T., *Am. J. Med.*, **8,** 565 (1950)
42. Paul, J. R., *Med. Bull. European Command*, U. S. Army, **8,** 445 (1951)
43. Paul, J. R., and Horstmann, D. M., *U. S. Armed Forces Med. J.*, **3,** 1457 (1952)

44. Warren, W. R., *U. S. Armed Forces Med. J.*, **4,** 313 (1953)
45. Gardner, H. T., *Am. J. Med.*, **8,** 561 (1950)
46. Burlingame, P. L., Gardner, H. T., Resemann, G., Cranmer, J., and Vollmer, E., *J. Lab. Clin. Med.*, **34,** 1284 (1949)
47. Gauld, R. L., *Am. J. Hyg.*, **43,** 248 (1946)
48. Havens, W. P., Jr., *U. S. Armed Forces Med. J.*, **3,** 1013 (1952)
49. Evans, A. S., *Proc. Soc. Exptl. Biol. Med.*, **75,** 809 (1950)
50. Ducci, H., Barahona, R., and Barzelatto, J., *Gastroenterology*, **17,** 45 (1951)
51. Sborov, V. M., and Keller, T. C., *Gastroenterology*, **19,** 424 (1951)
52. Opperman, A., and Wedekind, P., *Deut. med. Wochschr.* **75,** 1744 (1950)
53. Richards, H. J. A., *J. Roy. Army Med. Corps*, **95,** 103 (1950)
54. Pepper, B. O., *E. African Med. J.*, **27,** 317 (1950)
55. Snell, A. M., *GP*, **6,** 43 (1952)
56. Leibowitz, S., *Rev. Gastroenterol. N. Y.*, **16,** 721 (1949)
57. Thorling, L., *Acta Med. Scand.*, **137,** 322 (1950)
58. Leibowitz, S., and Gorman, W. F., *New Engl. J. Med.*, **246,** 932 (1952)
59. Wainwright, J., *Arch. Disease Childhood*, **25,** 286 (1950)
60. Wallgren, A., *Acta Med. Scand., Suppl. 246*, 251 (1950)
61. Lipman, B. L., *J. Am. Med. Assoc.*, **144,** 1090 (1950)
62. Mean, A., *Ann. Paediatr.*, **176,** 225 (1951)
63. Craig, J. M., and Landing, B. H., *Arch. Pathol.*, **54,** 321 (1952)
64. Drake, M., *Med. Clin. N. Amer.*, **36,** 1637 (1952)
65. Zimmerman, H. J., and Thomas, L. J., *Am. J. Med. Sci.*, **216,** 545 (1948)
66. Domenici, T. J., *New Engl. J. Med.*, **240,** 88 (1949)
67. Stahl, J., Levy, J. G., and Meschenmoser, *Strasbourg méd.*, **1,** 257 (1950)
68. Denber, H. C. B., and Leibowitz, S., *J. Am. Med. Assoc.*, **149,** 546 (1952)
69. Müller, T., *Schweiz. med. Wochschr.*, **77,** 796 (1947)
70. Salvesen, H. A., and Lödöen, O., *Acta Med. Scand.*, **137,** 305 (1950)
71. Swift, W. E., Jr., Gardner, H. T., Moore, D. J., Streitfeld, F. H., and Havens, W. P., Jr., *Am. J. Med.*, **8,** 614 (1950)
72. Koszalka, M. F., Lindert, M. C. F., Snodgrass, H. M., and Lerner, H. B., *Arch. Internal Med.*, **84,** 782 (1949)
73. Leibowitz, S., *J. Insurance Med.*, **5,** 10 (1950)
74. Kunkel, H. G., and Labby, D. H., *Ann. Internal Med.*, **32,** 433 (1950)
75. Zieve, L., Hill, E., Nesbitt, S., and Zieve, B., *Gastroenterology*, **25,** 495 (1953)
76. Post, J., Gellis, S., and Lindenauer, H. J., *Ann. Internal Med.*, **33,** 1378 (1950)
77. Gardner, H. T., Rovelstad, R. A., Moore, D. J., Streitfeld, F. H., and Knowlton, M., *Ann. Internal Med.*, **30,** 1009 (1949)
78. Havens, W. P., Jr., and Eichman, H. L., *J. Immunol.*, **64,** 349 (1950)
79. Havens, W. P., Jr., Lloyd, H., Melnick, J. L., and Colbert, J. W., Jr., *J. Infectious Diseases*, **85,** 139 (1949)
80. Evans, A. S., *J. Immunol.*, **64,** 411 (1950)
81. Leibowitz, S., *Am. J. Clin. Pathol.*, **21,** 201 (1951)
82. Pollard, M., and Bussell, R. H., *U. S. Air Force School of Aviation Medicine, Project No. 21-1401-0005, Rept. No. 3* (1953)
83. Ricketts, W. E., and Sterling, K., *J. Clin. Invest.*, **28,** 1477 (1949)
84. Rafsky, H. A., Weingarten, M., Krieger, C. I., Stern, K. G., and Newman, B., *Gastroenterology*, **14,** 29 (1950)

85. Popper, H., Bean, W. B., de la Huerga, J., Franklin, M., Tsumagari, Y., Routh, J. I., and Steigmann, F., *Gastroenterology*, **17,** 138 (1951)
86. Spellberg, M. A., Cohn, C., Wolfson, W. Q., and Shore, C., *Gastroenterology*, **14,** 11 (1950)
87. Martin, N. H., *Ciba Foundation Symposium on Liver Disease*, 68 (The Blakiston Co., Philadelphia, Pa., 249 pp., 1951)
88. Green, P., *Can. Med. Assoc. J.*, **63,** 365 (1950)
89. van Dommelen, C. K. V., and Francke, C., *Acta Med. Scand.*, **136,** 177 (1950)
90. Marrack, J. R., Johns, R. G. S., and Hoch, H., *Brit. J. Exptl. Pathol.*, **31,** 36 (1950)
91. Neefe, J. R., Gambescia, J. M., Gardner, H. T., and Knowlton, M., *Am. J. Med.*, **8,** 600 (1950)
92. Ducci, H., *Gastroenterology*, **15,** 628 (1950)
93. Maclagan, N. F., *Ciba Foundation Symposium on Liver Disease*, 1 (The Blakiston Co., Philadelphia, Pa., 249 pp., 1951)
94. Weller, S. D. V., *Great Ormond Street J.*, **1,** 26 (1951)
95. Armas-Cruz, R., Lobo-Parga, G., and Madrid, M., *J. Lab. Clin. Med.*, **39,** 533 (1952)
96. Hsia, D. Y. Y., and Gellis, S. S., *Am. J. Diseases Children*, **85,** 13 (1953)
97. Greenspan, E. M., Tepper, B., Terry, L. L., and Schoenbach, E. B., *J. Lab.Clin. Med.*, **39,** 44 (1952)
98. Havens, W. P., Jr., Eichman, H. L., and Knowlton, M., *Proc. Soc. Exptl. Biol. Med.*, **75,** 108 (1950)
99. Wood, H. F., and McCarty, M., *J. Clin. Invest.*, **30,** 616 (1951)
100. Gardner, H. T., Swift, W. E., Jr., Modica, M., Levintow, L., and Knowlton, M., *Am. J. Med.*, **8,** 584 (1950)
101. Albrink, M. J., Man, E. B., and Peters, J. P., *J. Clin. Invest.*, **29,** 781 (1950)
102. Vorhaus, L. J., Scudamore, H. H., and Kark, R. M., *Am. J. Med. Sci.*, **221,** 140 (1951)
103. Vorhaus, L. J., Scudamore, H. H., Gabuzda, G. J., Morey, G. R., Maloney, M. A., and Kark, R. M., *J. Lab. Clin. Med.*, **33,** 1475 (1948)
104. Ravin, H. A., Tsou, K. C., and Seligman, A. M., *J. Biol. Chem.*, **191,** 843 (1951)
105. Mann, J. D., Mandel, W. I., Eichman, P. L., Knowlton, M., and Sborov, V. M., *J. Lab. Clin. Med.*, **39,** 543 (1952)
106. Wilson, A., Calvert, R. J., and Geoghegan, H., *J. Clin. Invest.*, **31,** 815 (1952)
107. Peterson, R. E., *J. Lab. Clin. Med.*, **39,** 225 (1952)
108. Matassarin, B. M., and Delp, M. H., *Am. J. Med. Sci.*, **224,** 622 (1952)
109. Watson, C. J., Hawkinson, V., Capps, R. B., and Rappaport, E. M., *J. Clin. Invest.*, **28,** 621 (1949)
110. Sutherland, D. A., and Bollier, M., *Gastroenterology*, **20,** 39 (1952)
111. Havens, W. P., Jr., Miller, W. N., Swift, W. E., Jr., Gardner, H. T., and Knowlton, M., *Am. J. Med.*, **8,** 591 (1950)
112. Swift, W. E., Jr., Miller, W. N., Streitfeld, F. H., and Knowlton, M., *Am. J. Med.*, **8,** 581 (1950)
113. Kirsner, J. B., Sheffner, A. L., Palmer, W. L., and Bergeim, O., *J. Lab. Clin. Med.*, **36,** 735 (1950)
114. Dent, C. E., and Walshe, J. M., *Ciba Foundation Symposium on Liver Disease*, 22 (The Blakiston Co., Philadelphia, Pa., 249 pp., 1951)

115. Bjørneboe, M., Hamburger, C., and Jersild, M., *Acta Med. Scand.*, **136,** 287 (1950)
116. Nelson, R. S., Sprinz, H., Colbert, J. W., Jr., Cantrell, F. P., Havens, W. P., Jr., and Knowlton, M., *Am. J. Med.* **16,** 780 (1954)
117. Chalmers, T. C., Eckhardt, R. D., Reynolds, W. E., Cigarroa, J. G., Deane, N., Reifenstein, R. W., and Smith, C. W., *J. Clin. Invest.*, **32,** 559 (1953)
118. Campbell, R. E., and Pruitt, F. W., *Am. J. Med. Sci.*, **224,** 252 (1952)
119. Vieting, G., *Z. ges. inn. Med. u. ihre Grenzgebiete*, **5,** 657 (1950)
120. Davis, N., *Med. J. Australia*, **II,** 790 (1950)
121 Kipping, H., *Deut. med. Wochschr.*, **76,** 208 (1951)
122. Ström, J., *Brit. Med. J.*, **I,** 1168 (1950)
123. Lott, H., Young, R. W., and Stovall, J. C., *New Orleans Med. Surg. J.*, **102,** 528 (1950)
124. Shaffer, J. M., Farquhar, J. D., Stokes, J., Jr., and Sborov, V. M., *Am. J. Med. Sci.*, **220,** 1 (1950)
125. Rissel, E., *Helv. Med. Acta*, **17,** 404 (1950)
126. Hall, L., and Wharton, G. K., *Gastroenterology*, **19,** 69 (1951)
127. Lockwood, K. L., *J. Kentucky State Med. Assoc.*, **49,** 116 (1951)
128. Kalmansohn, R. W., Cleve, E. A., and Pruitt, F. W., *J. Philippine Med. Assoc.*, **27,** 549 (1951)
129. Colbert, J. W., Jr., Bungards, L., and Knowlton, M., *Proc. Soc. Exptl. Biol. Med.*, **79,** 339 (1952)
130. Farquhar, J. D., Stokes, J., Jr., Whitlock, C. M., Jr., Bluemle, L. W., Jr., and Gambescia, J. M., *Am. J. Med. Sci.*, **220,** 166 (1950)
131. McNeile, H. J., and Solomon, C., *N. Y. State J. Med.*, **50,** 1393 (1950)
132. Rhumball, J. M., Marion, D. F., and Sanders, M., *Gastroenterology*, **14,** 432 (1950)
133. Sborov, V. M., and Sutherland, D. A., *Gastroenterology*, **18,** 598 (1951)
134. Goldbloom, R. S., and Steigmann, F., *Gastroenterology*, **18,** 93 (1951)
135. György, P., Stokes, J., Jr., Smith, W. H., and Goldblatt, H., *Am. J. Med. Sci.*, **220,** 6 (1950)
136. Rivera, J. A., and Sborov, V. M., *Gastroenterology*, **17,** 546 (1951)
137. Sutherland, D. A., Mann, J. D., Giges, B., and Seligson, D., *Proc. Soc. Exptl. Biol. Med.*, **77,** 458 (1951)
138. Colbert, J. W., Jr., Holland, J. F., Heissler, I., and Knowlton, M., *Proc. 2nd Clin. ACTH Conf.*, **I,** 371 (1951)
139. Sborov, V. M., *Proc. 2nd Clin. ACTH Conf.*, **I,** 381 (1951)
140. Butt, H. R., Comfort, M. W., Power, M. H., and Mason, H. L., *J. Lab. Clin. Med.*, **37,** 870 (1951)
141. Colbert, J. W., Jr., Holland, J. F., Heissler, I., and Knowlton, M., *New Engl. J. Med.*, **245,** 172 (1951)
142. Rifkin, H., Marks, L. J., Hammerman, D. J., Blumenthal, M. J., Weiss, A., and Weingarten, B., *Arch. Internal Med.*, **89,** 32 (1952)
143. Evans, A. S., Sprinz, H., and Nelson, R. S., *Ann. Internal Med.*, **38,** 1115 (1953)
144. Evans, A. S., Sprinz, H., and Nelson, R. S., *Ann. Internal Med.*, **38,** 1134 (1953)
145. Ducci, H., and Katz, R., *Gastroenterology*, **21,** 357 (1952)
146. Evans, A. S., Sprinz, H., and Nelson, R. S., *Ann. Internal Med.*, **38,** 1148 (1953)
147. "The Problem of Homologous Serum Jaundice After Transfusions" (Editorial), *Gastroenterology*, **22,** 500 (1952)

148. Scheinberg, I. H., Kinney, T. D., and Janeway, C. A., *J. Am. Med. Assoc.*, **134,** 841 (1947)
149. McGraw, J. J., Jr., Strumia, M. M., and Burns, E., *Am. J. Clin. Pathol.*, **19,** 1004 (1949)
150. Ratnoff, O. D., and Mirick, G. S., *Bull. Johns Hopkins Hosp.*, **85,** 299 (1949)
151. Robinson, J. W., Twaddell, D. N., and Havens, W. P., Jr., *Ann. Internal Med.*, **32,** 1019 (1950)
152. Lehane, D., Kwantes, C. M. S., Upward, M. G., and Thomson, D. R., *Brit. Med. J.*, **II,** 572 (1949)
153. Corelli, F., del Greco, F., and Ruggieri, P., *Acta Med. Scand.*, **141,** 284 (1952)
154. Sborov, V. M., Giges, B., and Mann, J., *Arch. Internal Med.*, **92,** 678 (1953)
155. Kjaergaard, G., *Ugeskrift Laeger*, **112,** 1014 (1950)
156. Smith, M. H., and Hall, J. W., *J. Lab. Clin. Med.*, **33,** 998 (1948)
157. Koteen, H., Kane, C. A., and Rosenberg, M., *Am. J. Syphilis, Gonorrhea, Venereal Diseases*, **35,** 270 (1951)
158. Janeway, C. A., *J. Am. Med. Assoc.*, **138,** 859 (1948)
159. Lesses, M. F., and Hamolsky, M. W., *J. Am. Med. Assoc.*, **147,** 727 (1951)
160. Porter, J. E., *et al.*, *J. Am. Med. Assoc.*, **153,** 17 (1953)
161. Hsia, D. Y. Y., Kennell, J. H., and Gellis, S. S., *Am. J. Med. Sci.*, **226,** 261 (1953)
162. Paine, R. S., and Janeway, C. A., *J. Am. Med. Assoc.*, **150,** 199 (1952)
163. Murray, R., and Diefenbach, W. C. L., *Proc. Soc. Exptl. Biol. Med.*, **84,** 230 (1953)
164. Ordman, C. W., Jennings, C. G., Jr., and Janeway, C. A., *J. Clin. Invest.*, **23,** 541 (1944)
165. Cockburn, W. C., Harrington, J. A., Zeitlin, R. A., Morris, D., and Camps, F. E., *Brit. Med. J.*, **II,** 6 (1951)
166. Janeway, C. A., *Bull. N. Y. Acad. Med.*, **21,** 202 (1945)
167. Greenstein, N. M., Barenberg, L. H., and Steinberg, D., *Am. J. Diseases Children*, **81,** 218 (1951)
168. Barondess, J. A., *Pediatrics*, **10,** 732 (1952)
169. Odin, M., *Acta Med. Scand., Supplement 259*, 130 (1950)
170. Muri, J., *Tidsskr. Norske Laegeforen.*, **70,** 614 (1950)
171. Wilfuhr, G., *Deut. Gesundheitsw.*, **6,** 140 (1951)
172. Capps, R. B., Sborov, V., and Scheiffley, C. S., *J. Am. Med. Assoc.*, **136,** 819 (1948)
173. Borensztejn, D., *Lancet*, **I,** 941 (1948)
174. Benda, L., Rissel, E., and Thaler, H., *Deut. Arch. klin. Med.*, **197,** 477 (1950)
175. Arzt-Hofbauer, *Arch. Dermatol. u. Syphilis*, **191,** 350 (1950)
176. Franke, K., *Tuberkulosearzt*, **4,** 636 (1950)
177. Beyreder, J., *Wien. med. Wochschr.*, **100,** 447 (1950)
178. Sherwood, P. M., *Ann. Internal Med.*, **33,** 380 (1950)
179. Sauerwein, E., *Deut. zahnärztl. Z.*, **6,** 1306 (1951)
180. Nocke, E., *Zahnärztl. Rundschau*, **60,** 317 (1951)
181. Roberts, R. H., and Still, H., *Can. Med. Assoc. J.*, **62,** 75 (1950)
182. Smith, B. F., *J. Am. Med. Assoc.*, **144,** 1074 (1950)
183. Hobson, A. C. S., Fraser, D. E., and Newman, N. H., *Brit. Med. J.*, **I,** 1111 (1952)
184. Steigmann, F., Hyman, S., and Goldbloom, R., *Gastroenterology*, **15,** 642 (1950)
185. Appelbaum, E., and Kalkstein, M., *J. Am. Med. Assoc.*, **147,** 222 (1951)

186. Altschul, A., Foster, P. D., Paley, S. S., and Turner, L., *Arch. Internal Med.*, **89,** 24 (1952)
187. Cardon, P. V., and Beck, E. M., *N. Y. State J. Med.*, **52,** 1037 (1952)
188. Madsen, S., *Postgrad. Med.*, **11,** 517 (1952)
189. Gellis, S. S., *Am. J. Med. Technol.*, **17,** 293 (1951)
190. Kuh, C., and Ward, W. E., *J. Am. Med. Assoc.*, **143,** 631 (1950)
191. Trumbull, M. L., and Greiner, D. J., *J. Am. Med. Assoc.*, **145,** 965 (1951)
192. Leibowitz, S., Greenwald, L., Cohen, I., and Litwins, J., *J. Am. Med. Assoc.*, **140,** 1331 (1949)
193. Henle, W., Drake, M. E., Henle, G., and Stokes, J., Jr., *Archiv ges Virusforsch.*, **4,** 612 (1952)
194. McCollum, R. W., *Proc. Soc. Exptl. Biol. Med.*, **81,** 157 (1952)
195. Berk, J. E., Stokes, J., Jr., Drake, M., Malamut, L., Capps, R. B., and Henle, W., *Trans. & Studies, Coll. Physicians Phila.*, **19,** 151 (1952)
196. Neefe, J. R., Norris, R. F., Reinhold, J. G., Oliphant, J. W., Fitch, D. R., Watanabe, R. K., Mitchell, C. B., and Howell, D. S., *J. Clin. Invest.*, **31,** 652 (1952)
197. Stokes, J., Jr., *et al.*, *J. Am. Med. Assoc.* **154,** 1059 (1954)
198. Runyan, J., Wright, A. W., and Beebe, R. T., *J. Am. Med. Assoc.*, **144,** 1065 (1950)
199. Steele, H. H., *Gastroenterology*, **15,** 59 (1950)
200. Stokes, J., Jr., Blanchard, M., Neefe, J. R., Gellis, S. S., and Wade, G. R., *J. Am. Med. Assoc.*, **138,** 336 (1948)
201. Drake, M. E., Barondess, J. A., Bashe, W. J., Jr., Henle, G., Henle, W., Stokes, J., Jr., and Pennell, R. B., *J. Am. Med. Assoc.*, **152,** 690 (1953)
202. Oliphant, J. W., and Hollaender, A., *Public Health Repts.*, **61,** 598 (1946)
203. Blanchard, M. C., Stokes, J., Jr., Hampil, B., Wade, G. R., and Spizizen, J., *J. Am. Med. Assoc.*, **138,** 341 (1948)
204. Rosenthal, N., Bassen, F. A., and Michael, S. R., *J. Am. Med. Assoc.*, **144,** 224 (1950)
205. Barnett, R. N., Fox, R. A., and Snavely, J. G., *J. Am. Med. Assoc.*, **144,** 226 (1950)
206. James, G., Korns, R. F., and Wright, A. W., *J. Am. Med. Assoc.*, **144,** 228 (1950)
207. Lodmell, L. A., *J. Am. Med. Assoc.*, **147,** 1138 (1951)
208. Greenlee, R. G., Terrill, R. J., and Sloan, J. Q., *Texas State J. Med.*, **47,** 831 (1951)
209. Thompson, J. L., Jr., and Sutliff, W. D., *Southern Med. J.*, **45,** 801 (1952)
210. Albrecht, R. M., Korns, R. F., Beadenkopf, W. G., Goodman, M. B., Locke, F. B., and Marks, V., *J. Am. Med. Assoc.*, **152,** 1423 (1953)
211. Murphy, W. P., Jr., and Workman, W. G., *J. Am. Med. Assoc.*, **152,** 1421 (1953)
212. Hartmann, F. W., Mangun, G. H., Feeley, N., and Jackson, E., *Proc. Soc. Exptl. Biol. Med.*, **70,** 248 (1949)
213. Drake, M. E., Hampil, B., Pennell, R. B., Spizizen, J., Henle, W., and Stokes, J., Jr., *Proc. Soc. Exptl. Biol. Med.*, **80,** 310 (1952)
214. Allen, J. G., Sykes, C., Enerson, D. M., Moulder, P. V., Elghammer, R. M., Grossman, B. J., McKeen, C. L., and Galluzzi, N. J., *J. Am. Med. Assoc.*, **144,** 1069 (1950)
215. Allen, J. G., Enerson, D. M., Barron, E. S. G., and Sykes, C., *J. Am. Med. Assoc.*, **154,** 103 (1954)

216. Murray, R., cited by Allen, J. G., Enerson, D. M., Barron, E. S. G., and Sykes, C., *J. Am. Med. Assoc.* **154,** 103 (1954)
217. Strode, G. K., Bugher, J. C., Kerr, J. A., Smith, H. H., Smithburn, K. C., Taylor, R. M., Theiler, M., Warren, A. J., and Whitman, L., *Yellow Fever* (McGraw-Hill Book Co., Inc., New York, N. Y., 710 pp., 1951)
218. World Health Organization: Endemic yellow fever areas as delineated by WHO *Suppl. to Weekly Epidem. Record, R.E.H. No. 300* (1952); **27,** No. 39, 1–3 (Sept. 20, 1952)
219. Bevier, G., Tones Muñoz, N., and Doria-Medina, J., *Am. J. Trop. Med. Hyg.*, **2,** 464 (1953)
220. Gast Galvis, A., *Rev. fac. med. Univ. nac.* (*Bogotá*), **21,** 241 (1952)
221. Levi-Castillo, R., *Rev. Kuba med. trop. y parasitol.*, **8,** 76 (1952)
222. Levi-Castillo, R., *Z. Tropenmed. u. Parasitol.*, **4,** 254 (1953)
223. Garcia, E., *Bol. ofic. sanit. panamer.*, **31,** 340 (1951)
224. Clark, H. C., *Am. J. Trop. Med. Hyg.*, **1,** 78 (1952)
225. Soper, F. L., *Bol. ofic. sanit. panamer.*, **32,** 197 (1952)
226. de Caires, P. F., *West Indian Med. J.*, **1,** 3 (1951)
227. Blaksley, J. C., del Ponte, E., and Bejarano, J. F. R., *Bol. ofic. sanit. panamer.*, **31,** 39 (1951)
228. Kumm, H. W., *Trans. Roy. Soc. Trop. Med. Hyg.*, **43,** 673 (1950)
229. Komp, W. H. W., *Am. J. Trop. Med. Hyg.*, **1,** 330 (1952)
230. Kumm, H. W., *Am. J. Trop. Med. Hyg.*, **1,** 210 (1952)
231. Herrera, J. M., Elton, N. W., and Nicosia, J., *Arch. Hosp.* Santo Tomás, **4,** 1 (1949)
232. Elton, N. W., *Am. J. Public Health*, **42,** 170 (1952)
233. Elton, N. W., *Am. J. Public Health*, **42,** 1527 (1952)
234. Elton, N. W., *Military Surgeon*, **111,** 157 (1952)
235. Galindo, P., Trapido, H., and Carpenter, S. J., *Am. J. Trop. Med.*, **30,** 533 (1950)
236. Carpenter, S. J., Galindo, P., and Trapido, H., *Mosquito News*, **12,** 156 (1952)
237. Haddow, A. J., Dick, G. W. A., Lumsden, W. H. R., and Smithburn, K. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **45,** 189 (1951)
238. Lumsden, W. H. R., *Bull. Entomol. Research*, **42,** 317 (1951)
239. Dick, G. W. A., *Ann. Trop. Med. Parasitol.*, **44,** 319 (1950)
240. Dick, G. W. A., *Trans. Roy. Soc. Trop. Med. Hyg.*, **46,** 47 (1952)
241. de Rodaniche, E. C., *Am. J. Tropical Med. Hyg.*, **1,** 789 (1952)
242. Dick, G. W. A., and Gee, F., *Trans. Roy. Soc. Trop. Med. Hyg.*, **46,** 449 (1952)
243. Mathis, C., *Bull. acad. nat. méd.* (*Paris*), **134,** 644 (1950)
244. Macnamara, F. N., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47,** 199 (1953)
245. Stones, and Macnamara, F. N., cited by Dick, G. W. A., *Trop. Diseases Bull.*, **50,** 808 (1953)
246. Hahn, R. G., *Am. J. Hygiene*, **54,** 50 (1951)
247. Dick, G. W. A., *Am. J. Hygiene*, **55,** 140 (1952)
248. Dick, G. W. A., and Horgan, E. S., *J. Hyg.*, **50,** 376 (1952)
249. Hahn, R. G., and Bugher, J. C., *J. Immunol.*, **70,** 352 (1953)
250. Sabin, A. B., *Bact. Rev.*, **14,** 225 (1950)
251. Sabin, A. B., *Am. J. Trop. Med. Hyg.*, **1,** 30 (1952)
252. Sabin, A. B., and Schlesinger, R. W., *Science*, **101,** 640 (1945)
253. Sabin, A. B., and Young, I., *Proc. Soc. Exptl. Biol. Med.*, **69,** 478 (1948)
254. Reagan, R. L., and Brueckner, A. L., *J. Bact.*, **64,** 233 (1952)

255. Paul, J. R., Melnick, J. L., and Sabin, A. B., *Proc. Soc. Exptl. Biol. Med.*, **68**, 193 (1948)
256. Kimura, R., and Hotta, S., *Nippon Igaku Oyobi, Kenko-Hoken*, No. 3344, 1378 (1943)
257. Sabin, A. B., *Proc. 4th Intern. Congr. Trop. Med. Malaria*, **1**, 520 (1948)
258. Schlesinger, R. W., and Frankel, J. W., *Am. J. Trop. Med. Hyg.*, **1**, 66 (1952)
259. Meiklejohn, G., England, B., and Lennette, E. H., *Am. J. Trop. Med. Hyg.*, **1**, 51 (1952)
260. Meiklejohn, G., England, B., and Lennette, E. H., *Am. J. Trop. Med. Hyg.*, **1**, 59 (1952)
261. Reagan, R. L., and Brueckner, A. L., *J. Infectious Diseases*, **91**, 145 (1952)
262. Schlesinger, R. W., *Am. J. Hyg.*, **51**, 248 (1950)

ANTIGENIC VARIATION OF INFLUENZA VIRUSES[1,2]

By M. R. Hilleman

Department of Virus and Rickettsial Diseases, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

That viruses may undergo profound alteration in antigenic constitution has become increasingly apparent in recent years. Direct evidence of such change is exemplified by the modification which occurs naturally and in the course of laboratory manipulation of such genetically labile viruses as those of foot and mouth disease of cattle (1) and the influenza viruses of swine and man (see 2 to 6). Indirect support for this observation is the existence in nature of a multiplicity of closely related forms which fall into natural groups or families based on antigenic and other qualities. It seems reasonable to assume that the viruses included in the family of arthropod-borne encephalitic agents (7), for example, may have descended from a common ancestral parent through variation and selection in the course of residence in and passage through arthropod, avian, and mammalian hosts.

During the past two decades, a wealth of information concerning variation in human influenza viruses has been accumulated. These viruses have provided an excellent model for investigations of this sort for two principal reasons. First, the agents have a complex antigenic constitution, and they exhibit a degree of lability which, to present knowledge, has not been achieved by other viruses. Second, the cultivation of virus in embryonated eggs and the *in vitro* hemagglutination-inhibition test (8) have provided simple procedures for viral propagation and for measuring small antigenic differences between strains.

This review is limited to a selected bibliography of papers chosen to illustrate present concepts of antigenic variation of influenza viruses. A summation of viral alteration occurring in nature is given first and is followed by a consideration of recent studies in viral genetics which attempt to elucidate the mechanisms involved. Finally, the significance of immunological change as it relates to epidemiology and prevention of influenza is discussed.

VARIATION IN NATURE

The influenza viruses consist of three distinct immunological groups designated type A (including swine influenza), type B, and type C (9). The term A′ (10) has been applied to A-type viruses prevalent since 1946, which are markedly different antigenically from those which occurred previously.

[1] The survey of literature pertaining to this review was completed in December, 1953.

[2] The following abbreviations have been used in this chapter: O-D (refers to original and derived phase); WHO (World Health Organization).

Influenza A

Antigenic variation.—Historically, observations of antigenic differences among influenza viruses have been closely associated with attempts to classify these agents. Heterogeneity of antigenic constitution among strains isolated in different areas at different times was first recognized in 1936 by Magill & Francis (11). This observation was followed by attempts to segregate the strains into subgroups. Burnet in 1937 (12) studied antigenic variation in considerable detail and classified the influenza A viruses into three groups based on results of serum neutralization, cross-immunity, and serum absorption tests; these were designated New World, Old World, and Swine Influenza. Smith & Andrewes in 1938 (13) reported that four antigenic components of a group of A type viruses studied appeared to be of major importance. They divided the agents into three main groups of highly specific, relatively non-specific, and intermediate strains based on content of the four antigens. Francis & Magill (1938) (14, 15) did not believe the influenza A viruses could be separated with confidence into subtypes at that time. They emphasized the difficulty in assessing the significance of differences between strains so closely related and the inability to eliminate the problem of spontaneous variation in culture resulting from maintenance in the laboratory. These early investigations strongly suggested that the influenza agents comprised a mosaic of diverse antigens and that strain difference was based on qualitative and quantitative variation in these components.

During the early post-war period there was renewed interest in the determination of antigenic constitution of influenza viruses. These investigations were stimulated, largely, by the failure in 1947 (16 to 21) of the polyvalent vaccine to protect against the A′ viruses which became prevalent at that time. Further, the entrance of the World Health Organization (22) into the influenza field permitted an integrated study of this disease on a global basis for the first time and made strains available for study from all parts of the world.

Exact comparison of the results of the analyses made by different investigators is difficult because of employment of a wide variety of test methods and materials (for papers published since 1946 see 4, 5, 6, 21, 23 to 43). Thus, some of these workers used sera from virus-infected ferrets or hamsters, while others used sera prepared in chickens or rabbits given single or successive parenteral injections of virus. Some sera were processed to remove nonspecific inhibitors of hemagglutination (which may give false positive results in the tests), while others were not so treated. Most of the viruses isolated prior to 1945 were passed in ferrets and mice which, according to Hirst (44) and others (5, 45, 46), may result in marked alteration of antigenic constitution; the extent of influence of this factor upon the results cannot be readily assessed. Finally, the data of individual workers were subjected to different methods of analysis, presentation, and interpretation. It is of significance, however, that there is general agreement in the more important aspects.

There is a consensus that influenza A viruses isolated throughout the world in a given year tend to be homogeneous antigenically, although not necessarily identical. Further, there is progressive alteration of antigenic pattern of the prevalent viruses during successive epidemic years. Thus, antigenic complexes which were predominant in early years have been obscured or replaced by different components in more recent strains, and antigens dominant in the latter strains were not prominent in the early viruses. A relatively abrupt alteration in antigenic pattern appeared to occur in 1934 in the transition of the WS form of virus to PR8-like strains and again in 1946 when the A′ strains first became prevalent and seem to have replaced the agents related to PR8. Although several PR8-like viruses have been recovered since 1947, their origin from human patients or from laboratory contamination has been disputed (see 27, 28, 29, 37, 38, 43). Hilleman *et al.* (47) recently showed that the antibody level for PR8 virus in sera of children born since 1946 is very low, suggesting that infection with this agent, if present, is infrequent. Final proof for contemporary existence of agents of this form must await further isolations.

Van der Veen & Mulder (33) and Andrewes (48) on the basis of results of analyses with ferret antisera, concluded that the WS (1933), PR8-like (1934–1944), and A′ (1946–1953) strains were sufficiently different to constitute definite subgroups within the A type; individual differences within each subgroup were considered by Andrewes (48) to be but "variations on the main antigenic theme." Hilleman *et al.* (30, 34, 38) and Magill *et al.* (41), using chicken or rabbit antisera, found less evidence for this precise subgrouping. While the difference between WS, PR8, and A′ viruses was clear, the A type agents as a whole appeared to constitute a progressive series of antigenic forms, and the above subgroups merged into each other. Marked differences in antigenic pattern within the PR8-like and A′ subgroups were also evident.

The recent investigations by Isaacs and his associates (37, 40) have revealed two distinct subtypes among the A′ viruses recovered during 1950 to 1953 and tested with ferret antisera. These subtypes were designated Scandinavian (S) and Liverpool (L). Strains of the L subtype were very homogeneous antigenically while the S viruses were more heterogeneous. Among 96 strains recovered in 1950–1951, 46 were of S and 47 of L form. The 1952–1953 agents by contrast, were predominantly of S type (48).

It is of importance to note that while ferret antisera differentiated these subtypes, chicken and rabbit sera failed to do so (38, 40, 49). Indeed, studies by Hilleman (38; also unpublished observations) showed that strains isolated in 1950 and 1951 or 1952 and 1953 were strikingly homogeneous when tested with rooster antisera. This difference between animal sera may be associated, perhaps, with the fact that the ferret antibody resulted from "infection immunity" while the chicken antibody presumably did not. For studies aimed at detecting minor strain differences, as in epidemiological investigations, ferret antisera appear to have an advantage; on the other hand, where only the larger differences are important, as in investigations relating

to the prevalent viruses and the antigenic content of the vaccine, chicken or rabbit antisera appear best. The significance of L-S typing in studies of influenza epidemiology will be discussed later.

The results of recent strain analyses by Hirst (50) employing serum absorption procedures deserve separate consideration. Hirst absorbed influenza antisera with large amounts of virus and showed that antigens of the A viruses were of two kinds: group-specific antigens which convey the A character, and "type-specific" antigens peculiar to certain groups of strains. The large strain differences observed in tests with unabsorbed sera could generally be explained by qualitative variation in specific components. Where small differences occurred, the strains were often of the same type, and the differences could probably be explained by quantitative variation in antigenic content, especially the group antigen. Studies with specific absorbed sera against 10 A strains revealed seven distinct types. Sixty-one virus strains which were recovered between 1933 and 1951 were tested with these "type" sera. Based on his findings, Hirst concluded that the WS "type" disappeared in 1933 and the PR8 "type" in 1934. A Mel "type" was prevalent from 1935 to 1937, Ala-41 from 1935 to 1944, and Tal was found only in 1937. The FM1 "type" was prevalent from 1946 to 1950, and the status of 1950–1951 strains was unestablished. It is important to note that the viruses prevalent between 1934 and 1944 (PR8-like era) were not homogeneous; instead they were heterogeneous and comprised four distinct "types." Also, it is of interest that at least three different "types," Mel, Ala-41, and Tal occurred simultaneously in the 1935–1937 era. In discussing these conclusions, Hirst questioned his earlier assumption (44) that strain heterogeneity observed in the pre-1940 era was largely attributable to variation resulting from mouse adaptation; mouse adaptation was finally considered to induce small, but not large, changes in antigenic pattern without alteration of virus "type."

Jensen & Francis (51) also employed absorption techniques for antigenic analysis of these agents and detected 18 difference antigenic components among 29 influenza viruses studied. The 18 components were shared by strains recovered at widely separated intervals of time and it was concluded that "all type A strains probably possess the potential expression of each of these shared antigens, any of which might become strain dominant."

PQR variation.—Influenza viruses may exhibit peculiarities in serological behavior not associated with antigenic change. Thus, van der Veen and Mulder (33) described PQR variation among strains in their PR8-like or A′ subgroups tested with ferret antisera. The P viruses reacted to high titer in hemagglutination-inhibition tests with homologous sera only. Q strains were inhibited very poorly by homologous and all other sera, while the R viruses, which were few in number, were inhibited very well by all sera. The Q strains, in addition, generally stimulated poor antibody response in ferrets. The authors drew no particular significance from these observations other than to emphasize that the weak reactivity of Q strain antisera with homologous

virus and strong reactivity with heterologous R and some P viruses led to considerable difficulty in interpreting strain analysis data. Isaacs *et al.* (40) recently showed that PQ variation could be directed at will in the laboratory. Passage of a virus in P phase in eggs in the presence of graded amounts of homologous immune serum changed the agent into Q phase; Q virus, conversely, gained avidity for antibody on serial passage in normal mice, transforming to P phase.

Influenza B and C

The influenza B, like the A strains show diverse antigenic constitution (see 4, 6, 34, 38, 41, 48, 52 to 58). Most striking is the dissimilarity between Lee virus isolated in 1940 and the strains which occurred since 1943. While this difference is generally recognized, there is no consensus as to the extent of heterogeneity among the post-1943 strains. Brans (54), Bozzo (55), and Hennensen (56) found this latter group of viruses to be much alike even though recovered over a 9-year period. The findings of Hilleman *et al.* (34) agreed generally with this, although the existence of two subgroups among the post-1943 agents was suggested on the basis of minor antigenic differences. Tamm *et al.* (53), by contrast, concluded from their studies that the B strains, like influenza A, exhibit a "more or less continuous spectrum of antigenic differences." This general view was shared by Magill *et al.* (41) and by Jordan and his associates (58), who presented some indication for progressive alteration in a mathematical treatment of the data. It should be added that two additional strains (TM and Paddington isolated in 1940 and 1943 respectively), antigenically different from both Lee and the post-1943 viruses, have been recognized (54, 57).

Even though some heterogeneity is present among the B-type viruses, it is evident that these agents failed to show the marked antigenic lability observed among the A strains. This may be associated in some way with the greater endemicity of B-type infections compared with A.

Analyses by Francis *et al.* (9) and by Hilleman (38) have revealed antigenic homogeneity among the type C influenza viruses. However, the number of strains isolated to date is small, and final conclusions regarding the antigenic qualities of the group as a whole must await further study.

MECHANISM FOR VARIATION

Mutation, Variation, and Selection

For many years the view has been expressed that alteration of influenza virus in its natural environment is the result of selection of mutant variant forms which arise spontaneously. (Mutant here denotes an abrupt heritable change as contrasted to the lesser and more gradual changes referred to as variation.) While direct proof for this hypothesis is lacking, experimental findings strongly suggest that such mechanisms may be operative.

Mutation.—The most completely studied example of influenza virus

mutation is the "O-D"[2] change described by Burnet & his colleagues (59 to 63). This alteration was defined principally on the basis of differences in ability of A type viruses to propagate in the allantoic sac of embryonated eggs and to cause agglutination of fowl red blood cells. It was shown that virus recovered by injection of human throat washings into the amniotic cavity of embryonated eggs caused agglutination of human and guinea pig but not chicken erythrocytes and failed to grow in the allantoic sac. This virus, considered to be in "O" phase rapidly changed to "D" phase on amniotic passage, simultaneously acquiring the ability to propagate following allantoic inoculation and to agglutinate fowl erthrocytes. Fluids with virus in an intermediate phase were also described.

This phenomenon appeared to result from selection of spontaneous discontinuous mutants ("D") derived from the "O" form. The "D" virus was considered to have a potentially greater multiplication rate which permitted quick overgrowth of the "O" agent. "O-D" variation was estimated (60) to occur about once in every 10^5 or 10^6 new particles. "O" phase virus could be maintained without conversion to "D" by passage at low concentration in which the "D" mutants were diluted beyond probable occurrence. In further studies, Anderson & Burnet (62) showed that strains of virus recovered during interepidemic periods were converted more slowly to "D" phase than were epidemic strains. This suggested a possible relationship between viral mutability, expressed in terms of O-D variation, and ability to initiate an epidemic.

Magill & Sugg (64) confirmed the existence of "O" and "D" forms of virus but offered an alternative explanation for the mechanism involved. These workers reported that "O" or "D" virus might appear in egg passage in an abrupt and unpredictable manner following inoculation of one or the other viral phase. This was interpreted to indicate that "O-D" change was not the result of a discontinuous mutation; instead, it was a "reversible phenomenon associated with characteristics which seemed to be inherent in the virus particle." In a later paper (3, 65), Briody stated that the apparent change from "D" to "O" virus reported by Magill & Sugg did not constitute a valid argument against the discontinuous mutation theory. The presumed "O" virus of Magill & Sugg had only a single property in common with Burnet's "O" phase virus, viz., a similarity in ability to agglutinate fowl and guinea pig erythrocytes. The two "O" type viruses were clearly different in a number of other measurable properties. Briody has referred to the apparent "O" virus of Magill & Sugg as being in "XO" phase (pseudo O).

It is of some interest in this connection that the "O" or "D" phase properties of influenza viruses can be altered by changing the physical or chemical environment. Magill & Sugg (64) showed that fluids exhibiting "O" type agglutination could be induced to acquire "D" properties by simply lowering the pH of the system. Briody (66) found that heat, storage at 4°C. for several weeks, or the addition of inorganic ions to "O" viral antigens caused "D" agglutination. It is thus evident that alteration of environment may cause

changes in somatic qualities noninheritable (phenotypic), similar to those induced by genetic (genotypic) or other factors in egg passage.

Variation.—Adaptation of influenza virus to unusual host species, e.g., mice, may effect marked alteration in antigenic constitution of the agent. Hirst (44) showed that strains of influenza A, isolated in the chick embryo, multiplied readily in the lungs of mice and achieved an initial titer which was as high as ever obtained even after repeated passage. This high titer was unaccompanied either by lethal or visible pathogenic effects. On successive lung transfer in mice (from 4 to 5 passages), the agent became lethal in high dilution and caused extensive pulmonary consolidation, even though the capacity to multiply in mouse lung had not been augmented. The mouse adapted virus did, however, grow at a slightly faster rate than did the egg line virus. The process of mouse adaptation was simultaneously accompanied by alteration of viral antigenic pattern. Thus, two strains of virus initiated from the same egg line material changed on mouse passage along two divergent pathways so that they were not like each other and were not like the original starting strain.

Hirst did not refer to mutation in interpreting these data. Instead, he regarded the changes as resulting from selection of viral variants which differed from the bulk of the population by virtue of greater capacity to multiply in the new host. By serial mouse passage chance variants were selected and propagated until a fully adapted strain was evolved.

Sugg (45) confirmed Hirst's findings and showed that significant alteration of viral antigenic content was effected by 38 mouse transfers of the egg-adapted Cam (type A′) virus. It was further reported that another line of the Cam agent, passed only eight times in mice, was fully virulent for this species, even though the antigenic pattern had not been measurably altered. These results were interpreted by Sugg as indicating that antigenic change, which may occur during adaptation of an influenza virus to mice, is unrelated to the ability of virus to survive in the lung tissue or to acquire virulence for the mouse host. In discussing the mechanism for antigenic variation, Sugg pointed out that there was no adequate method available whereby cultures could be derived with certainty from a single virus particle. Therefore, it was stated,

> the possibility, however remote, must be considered that differences between two lines of influenza virus may be the result of a selection of different viruses present in the starting material rather than the result of variation in an originally homogeneous virus population.

It appears that egg passage of influenza virus may not result in the marked alteration of antigenic form such as accompanies mouse adaptation. This was first noted by Hirst (23), who emphasized the marked homogeneity evident when egg-adapted viruses have been used for comparison of strains from epidemics. More recently, Tamm *et al.* (67) showed that virus from eggs inoculated with human throat washings (first passage) did not differ

antigenically from later egg passage material, even though the strain had undergone rapid and profound alteration of "O-D" phase hemagglutinating properties between the first and second transfer. This evidence was used to support the assumption that strains recovered and passed in the chick embryo are comparable in antigenic composition to those causing disease in man.

Immunologic selection.—Antigenic alteration of influenza virus maintained in normal mice, such as just described, is accomplished in the absence of any detectable effect of immunological factors and may be presumed to result from nonspecific selective forces. Selection of variants on a specific immunological basis, by contrast, has been accomplished by Taylor (26) and by Archetti & Horsfall (68) and Archetti (69). These workers showed that antigenic variants emerged when strains of virus were passed serially *in ovo* in the presence of neutralizing antibody against a related but not identical strain. The variants possessed antigenic patterns different from those of the parent viruses and from those of the agents used to prepare the neutralizing antisera. Significant variation was clearly demonstrable (68, 69) after only three passages with antibody; the variant strains maintained the new antigenic pattern through at least 24 passages in the absence of antibody. It seemed that antigenic variants may be derived by this procedure from any strain of influenza A virus. An antigenic variant was obtained from the PR8 strain, which had been through hundreds of passages in several species since its recovery in 1934, with as much ease as from strains which had been through only a few passages in a single host.

Horsfall (5; see also 26) drew a parallel between these experimental results and the selective forces operative during natural transmission of influenza viruses in man. Viral variants possessing antigenic patterns which corresponded least with the neutralizing antibodies present in the partially immune hosts were considered to possess an immunological advantage, and their propagation was fostered. This mechanism for selection might account for the relatively small antigenic difference between strains recovered in a single epidemic year, and the more marked differences which are effected over a longer period of time.

Recombination

The preceding discussion of viral alteration by selection of variant and mutant forms has supposed that each virus particle is genetically isolated and that reproduction is exclusively vegetative. More recent studies [based largely on the results of earlier investigations of recombination in bacteriophage (see 70, 71)] have provided evidence that interchange of genetic qualities of two or more virus particles infecting the same host cell (recombination) may actually occur and result in mixing or transfer of antigenic and other factors.

The first investigations of genetic recombination of influenza viruses were carried out by Burnet and his associates in Australia (72 to 82). The descrip-

tion of a single experiment will illustrate their approach to the problem (78). The Mel and NWS strains were employed; both are type A viruses but are readily distinguishable antigenically. Further, the two agents behave differently in several *in vitro* serological tests, and only the NWS strain is pathogenic for mice by the cerebral route. In the experiment, mice were inoculated intracerebrally with large amounts of a mixture of the two viruses, and the animals were sacrificed four days later. Emulsions of the brains were titrated in the allantoic sac of embryonated eggs in the presence of anti-NWS ferret serum. Fluids from eggs infected at limiting dilution (i.e., the dilution of brain suspension which infected only part of the eggs and which was believed to contain but single virus particles) were tested for viral characteristics. Both Mel and NWS fluids, indistinguishable from the parent virus, were found. Some fluids were unique, however, in that the properties of both viruses were demonstrable. Thus, certain fluids contained virus which showed the antigenic pattern of Mel but possessed the neurotropic and other qualities of the NWS agent.

Later experiments (79 to 82) employing double infection of the allantoic cavity with the WSE and Mel agents gave two different recombinants designated Mel+ and WS−. Back recombination of the two "hybrid" strains (Mel+ and WS−) gave four kinds of fluids, i.e., the original parent WSE and Mel types plus the recombinant forms Mel+ and WS−. Fluids having one or another of the four forms (WSE, Mel, Mel+ or WS−) were not of equal number, as would be expected if each virus particle carried but a single genome. Instead, the distribution was such as to suggest that at least some of the virus particles carried more than one genome ("heterozygote"), and the data were consistent with there being, on the average, two virus particles each carrying two genomes to one carrying only a single genome.

Hirst & Gotlieb (83), reported successful combination of certain specific antigenic components from A type WSN and Mel viruses presumably into single virus particles. Strains WSN and Mel were different antigenically and could be distinguished readily in hemagglutination-inhibition tests with absorbed "monotypic" antisera. The two viruses were injected simultaneously in large amounts into the allantoic sacs of embryonated eggs and the fluids harvested 20 or 48 hr. later. Allantoic fluids from some of the eggs infected in this way yielded viral hemagglutinin which was inhibited by sera specific against each of the parent forms.

The reason for believing that actual combination had taken place was based on the prior observation that the hemagglutination-inhibition test will not detect more than one component in an artificial mixture of two antigenically distinct strains of virus. Thus, in mixtures containing near equivalent amounts of virus, no inhibition of hemagglutination will occur with antiserum to either strain. Where mixtures are not equivalent, the inhibition will be shown for the virus which is predominant. The fact that fluids from doubly-infected eggs were inhibited by antisera to both parent forms was

taken to indicate that the predominant virus in such fluids was constituted of the two antigens. This doubly-inhibitable hemagglutinin contributed as much as 84 per cent of the total hemagglutinin of certain fluids. Burnet (81) has also recorded observing fluids doubly-inhibitable by two antisera.

This particular experimental approach obviated the need for isolating pure clones of virus by limit dilution passage, a method considered unreliable by Hirst & Gotlieb (83) and by Liu & Henle (84) for separating mixtures.

In further experiments, Hirst & Gotlieb (85) showed that three varieties (X_1, X_2, and X_3) of combinant virus were produced on prolonged passage of the original fluids in eggs. X_1 occurred in ten passages and was considered capable of reversion to the parent form at a high rate. X_2 arose from X_1 but reverted more slowly to the WSN and Mel parent forms. X_1 and X_2 viruses were never obtained in pure culture. X_3 derived, after 32 passages, yielded only X_3 following egg inoculation and maintained this character through five limit dilution transfers. It was concluded the X_3 was a stable virus combining some of the specific antigenic components of each parent type and did not revert to the original forms. It should be pointed out that while combination of the phenotypic qualities of two viruses was produced, Hirst & Gotlieb drew no conclusions concerning the genetic mechanism involved.

Appleby (86) recently reported isolation of a probable recombinant of the A′ Kunz and neurotropic NWS viruses. This was accomplished by simultaneous inoculation of the two viruses (partially inactivated by ultraviolet light) intracerebrally into mice, followed five days later by passage of the brain suspension in eggs in the presence of NWS antiserum. The virus present in the allantoic fluid was neurotropic for mice (NWS character) but was neutralized by Kunz antiserum. It is of importance that the recombinant virus retained these properties for at least six intracerebral passages in mice, during which time the recombinant agent should have been freed of the nonneurotropic Kunz strain. This seemed to obviate the possibility of mixed infecting agents in the preparation.

Mechanism for viral recombination.—Attempts to explain the process by which viral recombination is effected are, of necessity, based on theories concerning the mechanism for viral reproduction.

It has been shown by Luria (87) and Luria & Dulbecco (88) that coli T bacteriophages, inactivated by ultraviolet light, still adsorb to bacteria. Infection of a bacterial cell by a single inactivated phage particle does not lead to viral multiplication; however, active phage propagation may follow multiple infection of the cell with these particles. This phenomenon was called "multiplicity reactivation." Luria & Dulbecco (88) made the assumption that there was inactivation of some but not all genetic units of the virus and that production of active phage from inactive resulted from recombination of "nonlethal" units to form active particles. To explain their hypothesis, these workers proposed that phage growth does not occur by binary fission but, instead, by independent reproduction of each genetic unit followed by reassembly of units from the genetic pool into complete phage

particles. This proposal was called the "recombination theory of multiplicity reactivation." In a subsequent critical analysis of the theory, Dulbecco (89) found certain discrepancies in the results which were not compatible with the recombination theory. It was concluded that the simple recombination hypothesis did not give a satisfactory interpretation of multiplicity reactivation; the observations of the phenomenon itself were not, however, invalidated.

Henle & Liu (90) produced evidence for multiplicity reactivation of influenza virus. Virus, partially inactivated by heat or ultraviolet light, propagated faster in growth curve experiments than could be accounted for on the basis of the amount of viable virus in the treated seed. Indication was given that reactivation occurred only when the host cell absorbed more than one noninfectious particle.

Shortly following Luria's hypothesis (87) of phage multiplication by replication of individual genetic units, Hoyle (91) suggested that influenza virus might be reproduced inside the cell in a similar fashion. According to Hoyle (91 to 95), the viral elementary body comprises a lipid envelope (derived from the host cell wall) which encloses a group of smaller units. These are (*a*) soluble group-specific complement-fixing antigen and (*b*) hemagglutinins (with enzymic qualities) which are the specific viral antigens. On entry of the virus particle into the host cell, the envelope is lost, releasing the smaller units. The soluble antigen is considered to be a self-reproducing ribonucleoprotein which multiplies in the cell giving rise, at a later stage, to hemagglutinins, the particular pattern being directed by the presence of some of the hemagglutinin from the original virus particle. Finally, the agglutinin and soluble antigen aggregate and destroy the mucoprotein of the cell wall. This results in mechanical weakening of the wall giving rise to protrusions containing viral substance. These protrusions break away from the cell yielding virus elementary bodies or filamentous forms.

Burnet (81) has proposed a modified theory for viral reproduction which is based largely on the ideas of Luria, Dulbecco, and Hoyle (see above). It was suggested that the soma of the virus is lost upon entry into the host cell and plays no further role. The individual genes of the viral genome (considered to be a linear aggregate of genes) are replicated, and this is followed by gene aggregation into a new genome. As soon as reconstituted, the genome creates a soma about itself and escapes through the cell wall which has likely been damaged. It was suggested that certain virus particles (including filamentous forms) may contain more than one complete genome, thereby accounting (as already discussed) for the apparent existence in recombination experiments of morphological units carrying the potentialities of more than one type of descendant.

Support for the hypothesis of independent replication of genetic units has been based on Hoyle's observation (91, 92, 93) of an orderly appearance of various viral properties in extracts of chorioallantoic membranes of eggs infected with large amounts of virus. Upon penetration of the cell, viral

infectivity was lost and did not appear again until 5 or 6 hr. later. Soluble antigen (ribonucleoprotein) however, increased to detectable levels in about 2 hr., and hemagglutinins were usually evident 1 hr. later. The early findings of Henle & Henle (96) agreed with Hoyle's observations. Subsequent studies by Kirber & Henle (97) revealed, however, that hemagglutinin actually appeared at the same time as soluble antigen; presence of the former component was made apparent by treating the membrane extract to remove a nonspecific inhibitor which prevented its detection. This finding was interpreted to indicate the presence of "immature" virus in the membrane extracts which gave complement-fixation and hemagglutination reactions but was noninfectious. The soluble antigen was not considered a virus precursor but, instead, a matrix substance in which the virus particles might be embedded in the host cell.

The recent publication by Fulton (98) provides an enlightening and critical analysis of the different theories of viral multiplication. In his own hypothesis, Fulton suggested that virus particles possess genes arranged in linkage groups and that such linkages are small in number but greater than one. Phage particles and linkage groups both multiply by binary fission, the formation of multinucleated cells being a distinct possibility. It was supposed that recombination is a common occurrence which normally takes place in the cycle following multiplication of the virus and is accomplished by fusion of two or more virus particles followed by segregation. Recombination together with point mutation was regarded as the cause of the phenotypic variation among virus particles. Soluble antigens were not considered to be virus precursors; instead they were assumed to derive from surface antigens of the particle by fragmentation and degradation or to represent matrix substance of viral origin surrounding the multiplying virus.

At the present time, direct evidence for the mechanism of viral reproduction is very meager, and the indirect evidence is, for the most part, equivocal. The theories which have been presented should be regarded as only working hypotheses which attempt to explain the experimental results or assist in the development of ideas for new experimental approaches. Final concepts must await more direct and definitive information.

SIGNIFICANCE OF VARIATION IN INFLUENZA VIRUSES

Epidemiology

Pandemic influenza.—Extreme variability in severity of clinical illness is one of the more prominent features of influenza and may be associated with genetic alterations in the virus. The most lethal form of the disease was manifest in the great pandemic of 1918–1919 (see 35, 48, 99 to 103), which is estimated to have caused the death of some 20 million persons. First indication of the pandemic was given in the late Spring of 1918 when there was a marked increase in cases of mild "3-day" influenza. The initial Spring wave was followed by two additional waves of the severe form of illness which

occurred in the Fall of 1918 and Spring of 1919 and which affected young adults of the 25- to 40-year age group primarily. Following the third wave, the disease gradually reverted to the ordinary pattern of low mortality directed principally toward aged persons.

It has been possible to explain the occurrence of epidemics of many diseases by an increase and decline in the proportion of susceptible individuals in the population. It would be tempting to use this kind of explanation for the generation of pandemic influenza were it not for the fact that pandemics have occurred 30 to 40 years apart, while individual immunity appears to be of relatively short duration. Under such circumstances, it would be difficult to explain why pandemics have not occurred more frequently. The failure of this classical hypothesis to explain the generation of pandemic influenza has led to the rather widely-accepted view that pandemic occurrence was dependent upon the marked change, (antigenic, virulence, or other) in a preexisting parasite and not upon some qualitative or quantitative alteration in the host population. It is the present consensus, though lacking in direct evidence, that the pandemic disease was caused by a virus and that it was similar biologically to the agents which are responsible for the present mild form of influenza. Thus, pandemic influenza has come to be regarded by many as epidemic influenza of unusual or unique character.

Burnet and his colleages (35, 99) have attempted to interpret the pandemic occurrence; this was, of necessity, speculative in the absence of knowledge concerning the etiological factor or factors involved. These workers suggested that the primary incitant was a type A influenza virus and that the pandemic onset was associated with a sharp change in genetic characteristics of the virus. The new kind of agent first appeared in Western France in the Spring of 1918 (following an epidemic of the usual form of influenza) where there was an intermingling of American soldiers and European service and civilian populations. It was suggested that the mixing of virus strains provided an opportunity for genetic recombination which resulted in a virus of greater virulence than could have arisen by a simple mutation. Further change in virulence in the pandemic was suggested by the increased severity of illness manifest during the occurrence in the Fall. The fact that exposure to the Spring epidemic offered protection to the severe Autumn wave in some areas, but not others, indicated that antigenic mutations may also have occurred during the progress of the pandemic. The recently expressed views of Andrewes (48) are in keeping with this general thesis.

Francis (103) recently postulated that essentially all the differences between the 1918–1919 pandemic and the epidemics of other years can be explained on the ground that the pandemic virus was of considerably greater virulence than epidemic strains. Marked alteration of viral antigenic characteristics was not considered necessary to explain the severity of the pandemic occurrence.

It was pointed out that persons in early life develop a highly strain-

specific immunity to the prevalent influenza viruses which declines during the middle years and is gradually replaced by a broad or composite immunity in later life resulting from repeated experience with these agents. Likewise, young people exhibit a high level of nonspecific physiologic tolerance to the effects of viral invasion which is considerably reduced in mid and late life.

According to Francis, the age incidence of epidemic influenza is a reflection of the immunity status of the individuals. Thus, influenza is most prevalent in 5- to 9-year-old persons in whom immunity is low; this is followed by a decline in cases among the 10- to 24-year group resulting from an increase in specific immunity. With loss of specific immunity in mid life, the incidence increases, and this is followed by a decline in the older age groups resulting from the development of composite immunity. This pattern of age-occurrence was essentially unchanged during the 1918–1919 pandemic except for an exaggerated decline in cases in older persons. This later finding was interpreted to indicate that the 1918 virus was not new antigenically. Instead it was a preexisting virus of exalted virulence.

The real difference between pandemic and epidemic influenza was in the high incidence of pneumonic complications and fatalities among persons of middle age (25 to 40) suffering from the pandemic disease. The lethal effect of the highly virulent pandemic virus was most marked in this group of individuals who had low specific and composite immunity and low physiologic ability to withstand the functional injury. The younger individuals were protected by higher specific immunity and by physiologic tolerance. Persons in the older age group escaped infection through effective acquired immunity (composite) to the virus. The 25 to 40 age group in 1918 was not selectively more susceptible to pneumonia than older persons; instead the latter individuals were less susceptible to influenza thereby escaping the viral injury which determined the likelihood of developing pneumonia and causing death. When infection did occur, older persons developed complications and fatal disease with the same frequency as middle aged persons.

Epidemic and endemic influenza.—Studies of influenza prevalence have clearly indicated a periodic recurrence of the disease in epidemic form. Influenza A epidemics tend to recur at 2- to 3-year intervals and influenza B at 4- to 6-year periods (102, 104, 105). No evidence has been presented to date to indicate a cyclic appearance of influenza C.

Much attention has been paid in recent years to considerations of the origin and cause for influenza epidemics and where the virus is maintained in the interepidemic periods (for discussion see 48, 100). Various possibilities have been suggested. One view is held that influenza viruses are always present in the community in sporadic or latent form and may be the source of virus for epidemics. Another view maintains that influenza may travel from country to country and is usually imported. Less likely, the virus may be maintained in animal reservoir species such as has been demonstrated for swine influenza by Shope (106). Another possibility, presented by Andrewes

(107), is that influenza virus may exist in several grades of virulence, epidemics arising through transformation from strains of virus of lower virulence. Finally, the antibody level in the host population may also play an important role in determining whether an epidemic may occur.

It became clearly evident some years ago that elucidation of the mechanism for origin and spread of influenza could be achieved best through a cooperative world-wide program for collection of epidemiologic and strain information. Accordingly, the World Health Organization (WHO) established an International Influenza Center in the laboratory of Dr. C. H. Andrewes in London for collection and analysis of viruses and data from regional laboratories and observation posts throughout the world (22). This program embodied many of the features of the system developed and employed by the U. S. Army Commission on Influenza (see 103, p. 208).

Even though the WHO[2] program has been operative only since 1947, much valuable information concerning epidemic spread of influenza has been collected to date. These findings have been summarized in a series of papers by Andrewes and his associates (32, 37, 40, 48, 101) at the World Influenza Center and deserve a brief review here.

The epidemic of 1948–1949 appeared to have its origin in a late summer outbreak (1948) in Sardinia, Sicily, and Calabria where the virus may have gone "underground" and become seeded in a widespread manner. In this same area, an epidemic of multicentric origin occurred in the Fall of 1948 and gradually spread northward through Europe to Sweden, where the force of the wave was spent. The strains isolated were antigenically homogeneous, and actual spread of the virus from country to country seemed likely.

There was essentially no influenza in Europe during the Winter of 1949 and 1950, but A′ virus became locally active in Sweden during the late Spring of 1950 and died down again. In the Autumn, influenza reappeared in the same area, followed by widespread epidemic prevalence throughout the world during the following Winter. For the first time, two antigenic "subtypes" of influenza A′ viruses were recognized in tests with ferret antisera termed S (Scandinavian) and L (Liverpool) (vide supra). The S virus, which originated in Sweden and Denmark, spread south and westward across Europe. The L agent, on the other hand, appeared to originate in the southern hemisphere (Australia and South Africa) from where it moved northward across Europe to England and caused a vicious type of illness in the Liverpool area resulting in the death of many aged and infirm individuals.

During the Winter of 1952-1953, influenza A′ was again prevalent, and the disease appeared almost simultaneously in Japan, the USA, France, and England. Later occurrence in more distant areas such as Iceland, Finland, Yugoslavia, Italy, and Portugal may have been the result of epidemic spread.

These studies suggested that influenza virus has two means for survival. First, it may exist in latent form and become widely seeded during the summer months of the year. Second, there may be a continuous series of infec-

tions in which epidemics occur in the southern hemisphere during the northern Summer travelling northward and causing infection in the northern hemisphere during the southern Summer, and vice versa. Studies by members of the Commission on Acute Respiratory Diseases of the U. S. Army (108) indicated that influenza virus may exist in endemic form in a single country (e.g., the U.S.A.) and does not need to go underground or travel back and forth across the equator in order to survive. Influenza A or B infection was shown to occur at some time during every month of the year over a 3½ year period. It was suggested that influenza A and B may be viewed as endemic diseases maintained by case to case transfer and periodically erupting into epidemic form.

While endemic disease does occur it seems that, in the main, epidemics must usually originate by country to country spread of virus. This seems the only reasonable means for explaining the striking antigenic homogeneity of viruses recovered throughout the World during a given year and the shift in antigenic pattern of strains in successive epidemic periods.

The views of Isaacs & Andrewes (37) relative to the role of PQ variation (*vide supra*) in survival of virus during interepidemic periods are of special interest. It was suggested that Q phase virus might be best able to survive in the partially-immune human population by virtue of its low avidity (affinity) for antibody. In support of this contention it was pointed out that nearly all of the S viruses isolated in the early outbreaks of 1950–1951 were in Q phase; P virus appeared late in the epidemic, presumably after a considerable number of viral transfers had been effected in the host population.

Prophylactic immunization.—Perhaps the most significant single aspect of antigenic variation in influenza from the practical sense is in its bearing on the problem of vaccination. This was forcibly demonstrated in the events concerning the 1947 vaccination trials. It had been shown previously that a polyvalent vaccine consisting of A type PR8 (1934) and Weiss (1943) and B type Lee (1940) viruses was highly effective in reducing influenza morbidity during the 1943–1944 A and the 1945–1946 B epidemics (see 6, 109). In 1947, however, the vaccine was completely ineffective (10, 16 to 21). The failure was shown to be a result of the marked difference between the A components of the vaccine and the A′ strains which caused the epidemic (6, 10, 16 to 21, 109). The natural sequence to the failure of 1947 has been the consideration of how to prevent another such occurrence. In essence, the problem is one of the limits to which influenza viruses can vary, and it may be considered in its two extremes.

There is the possibility, on the one hand, that influenza viruses are capable of almost limitless change. Horsfall (5) has pointed out that it is necessary to make only two assumptions to account for the origin and biological selection of variants of influenza viruses. These are:

(a) Viral variants arise spontaneously and continuously and (b) viral variants are selected for or against by environmental factors in the host which are operative during the multiplication process. If some new character provides a distinct preferential ad-

vantage for a variant during multiplication in a given host, it would be anticipated that eventually the variant should come to predominate and might actually eliminate the wild type of parent virus.

Under such circumstances, it is reasonable to look forward to relatively abrupt changes in viral antigenic pattern such as occurred in 1946-1947 as well as the more gradual alterations such as have occurred among the A′ strains during the period since 1947. This state of affairs was aptly summarized by Burent (35), who suggested that "influenza virus survives by constantly building serological novelty into its past antigenic structure."

The second possibility, discussed by Salk (109) and Francis (6), imposes a definite limit on the degree to which the antigenic constitution of influenza viruses may be altered. The observed differences have been interpreted by Salk to represent the

state of flux that is inevitable for a disease producing agent that is antigenically heterogeneous and consists of a family of organisms which may draw upon a genetic background of many antigenic components that can be forced to appear in one combination or another within the finite limits within which this agent can vary.

Thus,

during the interval when A′ strains are in the ascendancy and the type A strains seem to have disappeared, the immunity index to the type A members or constituents of the family will decline while the immunity to the A′ components will increase. When the immunity to the A′ strains is sufficiently high and the level of population immunity to type A strains is sufficiently low, one might expect that the A′ group will be replaced by the A once again.

Whether the limits of influenza virus variation are finite (implying a small number) or not finite (implying a large number) is of great importance, since it embraces the question of whether a single vaccine can eventually be prepared which will protect against any and all strains of virus. Salk (109), in adhering to the hypothesis of finite variation, is seeking to assemble a group of strains which, in composite, will include all the antigenic forms of influenza virus. According to this idea, once all the antigenic varieties of influenza have been captured and included in the vaccine, it should be possible to provide a significant immunity index to all the possible viral antigenic combinations.

The recent developments by Salk *et al.* (110 to 113) in studies with immunologic adjuvants may partially, at least, deemphasize the importance of strain variation in relation to the vaccination problem. It was shown that virus vaccine incorporated in an oily emulsion (mineral oil with an emulsifying agent, Arlacel A) gave antibody levels much higher and longer sustained than when the ordinary aqueous preparation was employed. Further, a considerably broader immunologic response was obtained to heterologous related strains following injection of a single virus.

It seems reasonable to assume that the driving forces of variation and selection may cause an alternate regression and egression of existing anti-

genic components and thus account for perhaps the majority of antigenic changes seen during the contemporary era. Under these circumstances, the attempt to prepare a polyvalent vaccine comprising all the known antigens of influenza appears to constitute a sound approach to the problem of prophylactic immunization. It seems equally likely, however, that the forces of mutation and selection may cause the appearance of new antigens which never occurred previously and that such changes might be simultaneously accompanied by enhanced virulence for man. Faced with this possibility of the gradual or abrupt appearance of new virus forms, it may be necessary for some time to keep a close watch on the antigenic constitution of strains as they occur, and to incorporate the new ones into the vaccine as appropriate. The efforts of the world-wide network of influenza outposts coordinated by the World Health Organization should go far toward detecting the new and unique virus when it first occurs, thereby permitting its incorporation into the vaccine in advance of the more wide spread occurrence.

LITERATURE CITED

1. Galloway, I. A., Henderson, W. M., and Brooksby, J. B., *Proc. Soc. Exptl. Biol. Med.*, **69**, 57–63 (1948)
2. Burnet, F. M., in *Handbuch der Virusforschung*, Suppl. **II**, 47–65 (Doerr, R., and Hallauer, C., Eds., Springer-Verlag, Wien, Austria, 425 pp., 1950)
3. Briody, B. A., *Bact. Revs.*, **14**, 65–95 (1950)
4. Hilleman, M. R., *Federation Proc.*, **11**, 798–803 (1952)
5. Horsfall, F. L., Jr., *Federation Proc.*, **11**, 804–7 (1952)
6. Francis, T., Jr., *Federation Proc.*, **11**, 808–12 (1952)
7. Hammon, W. McD., *Am. J. Trop. Med.*, **28**, 515–25 (1948)
8. Hirst, G. K., *J. Exptl. Med.*, **75**, 49–64 (1942)
9. Francis, T., Jr., Quilligan, J. J., Jr., and Minuse, E., *Science*, **112**, 495–97 (1950)
10. Salk, J. E., and Suriano, P. C., *Am. J. Public Health*, **39**, 345–55 (1949)
11. Magill, T. P., and Francis, T., Jr., *Proc. Soc. Exptl. Biol. Med.*, **35**, 463–66 (1936)
12. Burnet, F. M., *Australian J. Exptl. Biol. Med. Sci.*, **15**, 369–74(1937)
13. Smith, W., and Andrewes, C. H., *Brit. J. Exptl. Pathol.*, **19**, 293–314 (1938)
14. Magill, T. P., and Francis, T., Jr., *Brit. J. Exptl. Pathol.*, **19**, 273–84 (1938)
15. Francis, T., Jr., and Magill, T. P., *Brit. J. Exptl. Pathol.*, **19**, 284–93 (1938)
16. Francis, T., Jr., Salk, J. E., and Quilligan, J. J., Jr., *Am. J. Public Health*, **37**, 1013–16 (1947)
17. Loosli, C. G., Schoenberger, J., and Barnett, G., *J. Lab. Clin. Med.*, **33**, 789–98 (1948)
18. Mellanby, H., Andrewes, C. H., Dudgeon, J. A., and MacKay, D. G., *Lancet*, **I**, 978–82 (1948)
19. Dudgeon, J. A., Mellanby, H., Glover, R. E., and Andrewes, C. H., *Brit. J. Exptl. Pathol.*, **29**, 132–41 (1948)
20. Sigel, M. M., Shaffer, F. W., Kirber, M. W., Light, A. B., and Henle, W., *J. Am. Med. Assoc.*, **136**, 437–41 (1948)
21. Rasmussen, A. F., Jr., Stokes, J. C., Smadel, J. E., *Am. J. Hyg.*, **47**, 142–49 (1948)
22. Payne, A. M. M., *Bull. World Health Organization*, **8**, 755–74 (1953)
23. Hirst, G. K., *J. Exptl. Med.*, **86**, 367–81 (1947)
24. Anderson, S. G., *Australian J. Sci.*, **11**, 59 (1948)
25. Andrewes, C. H., *Proc. Roy. Soc. Med.*, **42**, 519–22 (1949)
26. Taylor, R. M., *Am. J. Public Health*, **39**, 171–78 (1949)
27. Lepine, P., Sautter, V., Reinie, L., and Maurin, J., *Ann. inst. Pasteur*, **77**, 108–26 (1949)
28. van Rooyen, C. E., McClelland, L., and Campbell, E. K., *Can. J. Pub. Health*, **40**, 447–56 (1949)
29. Nagler, F. P., van Rooyen, C. E., and Sturdy, J. H., *Can. J. Pub. Health*, **40**, 457–65 (1949)
30. Hilleman, M. R., Mason, R. P., and Rogers, N. G., *Public Health Repts. (U.S.)*, **65**, 771–77 (1950)
31. Appleby, J. C., and Stuart-Harris, C. H., *Brit. J. Exptl. Pathol.*, **31**, 797–805 (1950)
32. Chu, C. M., Andrewes, C. H., and Gledhill, A. W., *Bull. World Health Organization*, **3**, 187–214 (1950)
33. van der Veen, J., and Mulder, J., *Studies on the Antigenic Composition of Human Influenza Virus A Strains with the Aid of the Haemagglutination Inhibition*

Technique, Onderzoekingen en Mededelingen Uit het Instituut voor Praeventieve Geneeskunde, No. 6 (Stenfert-Kroese, Leiden, Netherlands, 65 pp., 1950)

34. Hilleman, M. R., Mason, R. P., and Buescher, E. L., *Proc. Soc. Exptl. Biol. Med.*, **75**, 829–35 (1950)
35. Burnet, F. M., *Bull. Johns Hopkins Hosp.*, **88**, 157–80 (1951)
36. Smith, W., Westwood, J. C. N., and Belyavin, G., *Lancet*, **II**, 1189–93 (1951)
37. Isaacs, A., and Andrewes, C. H., *Brit. Med. J.*, **II**, 921–27 (1951)
38. Hilleman, M. R., *Proc. Soc. Exptl. Biol. Med.*, **78**, 208–15 (1951)
39. Isaacs, A., *Proc. Roy. Soc. Med.*, **44**, 801–3 (1951)
40. Isaacs, A., Gledhill, A. W., and Andrewes, C. H., *Bull. World Health Organization*, **6**, 287–315 (1952)
41. Magill, T. P., and Jotz, A. C., *J. Bact.*, **64**, 619–28 (1952)
42. Hilleman, M. R., and Horsfall, F. L., Jr., *J. Immunol.*, **69**, 343–356 (1952)
43. Dempster, G., and Buchner, B., *Can. Med. Assoc., J.*, **69**, 119–24 (1953)
44. Hirst, G. K., *J. Exptl. Med.*, **86**, 357–66 (1947)
45. Sugg, J. Y., *J. Bact.*, **58**, 399–406 (1949)
46. Mulder, J., and Brans, L. M., *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **18**, 139–52 (1952)
47. Hilleman, M. R., Werner, J. H., and Gauld, R. L., *Bull. World Health Organization*, **8**, 613–31 (1953)
48. Andrewes, C. H., *Bull. World Health Organization*, **8**, 595–612 (1953)
49. Sampaio, A. A. C., *Bull. World Health Organization*, **6**, 473–80 (1952)
50. Hirst, G. K., *J. Exptl. Med.*, **96**, 589–603 (1952)
51. Jensen, K. E., and Francis, T., Jr., *J. Exptl. Med.*, **98**, 619–39 (1953)
52. Lazarus, A. S., *Proc. Soc. Exptl. Biol. Med.*, **71**, 317–19 (1949)
53. Tamm, I., Kilbourne, E. D., and Horsfall, F. L., Jr., *Proc. Soc. Exptl. Biol. Med.*, **75**, 89–92 (1950)
54. Brans, L. M., *Studies on the Antigenic Composition of Influenza Virus B Strains with the Aid of the Haemagglutinatoin-Inhibition Technique*, Onderzoekingen en Mededelingen Uit het Instituut voor Praeventieve Geneeskunde, No. 9 (Stenfert-Kroese, Leiden, Netherlands, 32 pp., 1952)
55. Bozzo, A., *Bull. World Health Organization*, **5**, 149–63 (1952)
56. Hennensen, W. A., *Bull. World Health Organization*, **6**, 481–85 (1952)
57. Mulder, J., Brans, L. M., and Nooyer, I. De., *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **19**, 189–96 (1953)
58. Jordan, W. S., Jr., and Gaylin, S. G., *J. Immunol.*, **70**, 393–99 (1953)
59. Burnet, F. M., *Australian J. Sci.*, **5**, 81–83 (1942)
60. Burnet, F. M., and Bull, D. R., *Australian J. Exptl. Biol. Med. Sci.*, **21**, 55–69 (1943)
61. Burnet, F. M., and Stone, J. D., *Australian J. Exptl. Biol. Med., Sci.*, **23**, 151–60 (1945)
62. Anderson, S. G., and Burnet, F. M., *Australian J. Exptl. Biol. Med. Sci.*, **25**, 235–42 (1947)
63. Burnet, F. M., *Bull. Johns Hopkins Hosp.*, **88**, 137–57 (1951)
64. Magill, T. P., and Sugg, J. Y., *J. Exptl. Med.*, **87**, 535–46 (1948)
65. Briody, B. A., *J. Infectious Diseases*, **83**, 293–98 (1948)
66. Briody, B. A., *J. Infectious Diseases*, **83**, 283–87 (1948)

67. Tamm, I., Ginsberg, H. S., and Horsfall, F. L., Jr., *Proc. Soc. Exptl. Biol. Med.*, **81**, 94–98 (1952)
68. Archetti, I., and Horsfall, F. L., Jr., *J. Exptl. Med.*, **92**, 441–62 (1950)
69. Archetti, I., *Proc. Soc. Exptl. Biol. Med.*, **80**, 212–14 (1952)
70. Hershey, A. D., and Rotman, R., *Genetics*, **34**, 44–71 (1949)
71. Hershey, A. D., and Chase, M., *Cold Spring Harbor Symposia Quant. Biol.*, **16**, 471-79 (1951)
72. Burnet, F. M., and Lind, P. E., *Australian J. Sci.*, **12**, 109–10 (1949)
73. Burnet, F. M., *Trans. N. Y. Acad. Sci.*, **13**, 2–4 (1950)
74. Burnet, F. M., *J. Gen. Microbiol.*, **5**, 46–53 (1951)
75. Burnet, F. M., *J. Gen. Microbiol.*, **5**, 54–58 (1951)
76. Burnet, F. M., and Lind, P. E., *J. Gen. Microbiol.*, **5**, 59–66 (1951)
77. Burnet, F. M., and Lind, P. E., *J. Gen. Microbiol.*, **5**, 67–82 (1951)
78. Burnet, F. M., and Edney, M., *Australian J. Exptl. Biol. Med. Sci.*, **29**, 353–62 (1951)
79. Burnet, F. M., and Lind, P. E., *Australian J. Exptl. Biol. Med. Sci.*, **30**, 469–77 (1952)
80. Burnet, McF., Fraser, K. B., and Lind, P. E., *Nature*, **171**, 163–65 (1953)
81. Burnet, McF., *Bull. World Health Organization*, **8**, 661–82 (1953)
82. Lind, P. E., and Burnet, F. M., *Australian J. Exptl. Biol. Med. Sci.*, **31**, 361–72 (1953)
83. Hirst, G. K., and Gotlieb, T., *J. Exptl. Med.*, **98**, 41–52 (1953)
84. Liu, O. C., and Henle, W., *J. Exptl. Med.*, **97**, 889–902 (1953)
85. Hirst, G. K., and Gotlieb, T., *J. Exptl. Med.*, **98**, 53–70 (1953)
86. Appleby, J. C., *Brit. J. Exptl. Pathol.*, **33**, 280–87 (1952)
87. Luria, S. E., *Proc. Natl. Acad. Sci. U. S.*, **33**, 253–64 (1947)
88. Luria, S. E., and Dulbecco, R., *Genetics*, **34**, 93–125 (1949)
89. Dulbecco, R., *J. Bact.*, **63**, 199–207 (1952)
90. Henle, W., and Liu, O. C., *J. Exptl. Med.*, **94**, 305–22 (1951)
91. Hoyle, L., *Brit. J. Exptl. Pathol.*, **29**, 390–99 (1948)
92. Hoyle, L., *J. Hyg.*, **48**, 277–97 (1950)
93. Hoyle, L., *J. Hyg.*, **50**, 229–45 (1952)
94. Hoyle, L., in *The Nature of Virus Multiplication* 225–48 (Fildes, P., and van Heyningen, W. E., Cambridge University Press, Cambridge, England, 320 pp., 1953)
95. Hoyle, L., Reed, R., and Astbury, W. T., *Nature*, **171**, 256–57 (1953)
96. Henle, W., and Henle, G., *J. Exptl. Med.*, **90**, 23–37 (1949)
97. Kirber, M. W., and Henle, W., *J. Immunol.*, **65**, 229–44 (1950)
98. Fulton, F., in *The Nature of Virus Multiplication*, 261–75 (Fildes, P., and van Heyningen, W. E., Cambridge University Press, Cambridge, England, 320 pp., 1953)
99. Burnet, F. M., and Clark, E., *Influenza: A Survey of the Last 50 Years in the Light of Modern Work on the Virus of Epidemic Influenza*, Monograph 4, Walter and Eliza Hall Inst. Research in Pathol. and Med. (MacMillan and Co., Ltd., London, England, 118 pp., 1942)
100. van Rooyen, C. E., and Rhodes, A. J., *Virus Diseases of Man*, 561–86 (Thomas Nelson and Sons, New York, N. Y., 1202 pp., 1948)
101. Andrewes, C. H., *New Engl. J. Med.*, **242**, 197–203 (1950)

102. Stuart-Harris, C. H., *Influenza and other Virus Infections of the Respiratory Tract* (Edward Arnold and Co., London, England, 235 pp., 1953)
103. Francis, T., Jr., *Ann. Internal Med.*, **39**, 203–21 (1953)
104. Commission on Acute Respiratory Diseases, U. S. Army, *Am. J. Hyg.*, **43**, 29–37 (1946)
105. Collins, S. D., and Lehmann, J., *Public Health Repts. (U. S.)*, **66**, 1487–1516 (1951)
106. Shope, R. E., *J. Exptl. Med.*, **77**, 127–38 (1943)
107. Andrewes, C. H., *Proc. Roy. Soc. Med.*, **36**, 1–10 (1942)
108. Commission on Acute Respiratory Diseases, U. S. Army, *Am. J. Hyg.*, **47**, 290–96 (1948)
109. Salk, J. E., *Bull. N. Y. Acad. Med.*, **28**, 748–65 (1952)
110. Salk, J. E., Laurent, A. M., and Bailey, M. L., *Am. J. Public Health*, **41**, 669–77 (1951)
111. Salk, J. E., and Laurent, A. M., *J. Exptl. Med.*, **95**, 429–47(1952)
112. Salk, J. E., Bailey, M. L., and Laurent, A. M., *Am. J. Hyg.*, **55**, 439–56 (1952)
113. Salk, J. E., *J. Am. Med. Assoc.*, **151**, 1169–75 (1953)

LABELLED ANTIGENS AND ANTIBODIES[1,2]

By Albert H. Coons[3]

Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts

The thorough-going survey of the recent immunological literature prepared by Haurowitz for the preceding volume of this series, in which were included papers dealing with the use of labelled reagents in immunology up to the end of 1952, makes a complete listing redundant. Moreover, choosing a tactic rather than a problem leads into far by-ways. For these reasons some excellent contributions will not be dealt with. Rather, a somewhat more leisurely consideration will be given to the fate of antigens and antibodies after injection into the animal, a problem of lasting importance to the immunologist, and one which can only be approached (at least at present) with labelled immunological reagents.

The problems which have been studied by means of labelled antigens and antibodies are the cellular distribution of injected antigenic substances, their persistence in the blood and tissues, the effect of antibody on their persistence, the nature of the degradation-products, and, by inference, the sites of antibody formation and its mechanism. For comparison, a limited number of studies have been carried out on similar nonantigenic molecules, i.e., homologous proteins.

A Consideration of Labels

The ideal label would allow the tracing of the property under study. In the case of antigens this means that the label should not alter the physical properties, the susceptibility to enzymatic degradation, or the antigenic activity of the labelled substance. It should have no effect of its own on tissue cells. In order that the tracing be precise, the label should drop out of the molecule and diffuse rapidly away when the labelled antigen has been so degraded or otherwise altered *in vivo* that it is no longer antigenically active. Finally, it should be easily quantitated and so distinctive as to allow the recognition of a single molecule in a precise locus in a single cell. Most of these requirements, in somewhat different form, have been stated by Ingraham (1). Needless to say, there is no such label, but it is useful to have an ideal label in mind in order to be clearly aware of the relative advantages and limitations of a real label in a given circumstance.

[1] The survey of literature pertaining to this review was completed in December, 1953, but a few more papers have been cited.

[2] The following abbreviations have been used in this chapter: ADR (Antigen disappearance rate).

[3] This review was written during tenure of a Career Investigatorship of the American Heart Association.

So far in the development of such studies there have been two kinds of labels employed, visible and isotopic, and two ways of employing them. The most extensively used tactic has been to label the antigenic molecule and to follow the label after injection. Another approach has been a histochemical one, using visibly-labelled antibody applied to tissue sections *in vitro* to detect antigen previously injected.

Iodine—131.—Since proteins lightly labelled with radioactive halogen atoms have been those most extensively studied, the evidence bearing on the evaluation of these labels will be presented first, and other labelling materials compared with them. Fine & Seligman (2) were the first to use radioactive halogen atoms to label proteins. They labelled canine plasma proteins with Br^{82} and subjected the product to hydrolysis with trypsin or with sulfuric acid. In each case the increase in concentration of bromide ion which was no longer precipitable with trichloracetic acid amounted to about 2 per cent. Similar amounts of bromide were found in the plasma of dogs 12 hr. after the injection of bromoproteins. They reached the tentative conclusion that 97 per cent of the bromine in such a linkage with tyrosine and possibly tryptophan [Wormall, 1930 (3)] "would be liberated by nothing less than destruction or degradation of the aromatic ring holding the bromine atom." On comparison with S^{35}-labelled homologous plasma proteins (produced by feeding S^{35}-containing cystine, homocystine, or methionine to dogs and harvesting the plasma), brominated proteins had a comparable though somewhat faster disappearance rate from the circulation after injection provided they contained not more than from 0.1 to 0.2 per cent bromine (one atom/molecule). With increasing bromination there was faster and faster disappearance from the blood. Finally, they found that more rapid disappearance was associated with denaturation resulting from bromination at high pH values. Similar experiments with I^{131} indicated that this atom behaved like Br^{82} on alkaline enzymatic hydrolysis but that its bond was considerably less stable in strong acid (4). Subsequent work has confirmed and extended these conclusions. Following the demonstration by Eisen & Keston (5) that lightly iodinated bovine serum albumin (one I^{131} atom/10 protein molecules) could not be distinguished immunochemically from unlabelled antigen, Knox & Endicott (6) studied the disappearance of native and I^{131}-labelled (slightly less than 1 atom/molecule) bovine serum albumin from the circulation of rabbits after the intravenous injection of doses of about 25 mg. of antigen N. The circulating antigen level was traced by determinations of antigen N and by measurements of radioactivity, providing a test of the adequacy of the label. Their results, as illustrated in Table I, indicated that there was no difference in the disappearance of antigen as determined by its antigenic activity or by radioactivity, nor was there any difference between the disappearance of labelled and unlabelled bovine serum albumin, until the seventh day. After this, the beginning of Phase C (see below) was delayed by one day in the case of the iodinated albumin.

Similar results were obtained in guinea pigs from a somewhat different

TABLE I

COMPARISON OF ANTIGEN N IN THE CIRCULATING SERUM

Micrograms of N per gram of serum determined by the quantitative precipitin tests (ppt) and by radioactivity measurements (I^{131}) following intravenously administered $BSaI^{131}$*

Time†	Rabbit #5		Rabbit #6		Rabbit #7		Rabbit #8		Rabbit #9		Rabbit #10	
	ppt	I^{131}	ppt	I^{131}	ppt	I^{131}	ppt	I^{131}	ppt	I^{131}	ppt	I^{131}
0.005	210	200	270	260	260	270	190	180	240	220	210	210
0.13	160	150	200	190	170	170	130	120	150	150	160	150
0.37	120	110	150	140	130	130	100	90	120	120	83	110
1.2	73	78	120	110	99	92	65	65	73	83	81	82
2.2		62	87	79	76	73	55	55	64	68	64	67
3.1	62	59	67	68	65	66		49	64	61		59
4.2	49	49		58	52	53	55	51	52	54	46	47
5.2	52	49	57	53		48	47	46	48	46	30	43
6.1	39	38	44	44	43							
7.3	‡							32		35	18	
8.1			27	23		22						
9.3							20	4.0	32	30	−Ag	
											+Ab	4.0
10.1			−Ag		−Ag							
			+Ab	0.4	+Ab	5.0						
11.4							−Ag				−Ag	
							+Ab	3.0		20	+Ab	5.0
12.3			−Ag		−Ag							
			+Ab	0.2	+Ab	5.0						

* From Knox & Endicott (6).

† Time in days after injection time. Animals were bled at slightly different intervals, hence times are averages, accurate to about 0.1 day after first day.

‡ Rabbit #5 died following cardiac puncture on 6th day.

approach by Masouredis, Melcher, & Koblick (7), and by Melcher & Masouredis (8). They marked antihuman serum albumin rabbit globulin with I^{131}. Their careful experiments indicated that the *in vitro* behavior of the antibody was unchanged by the labelling procedure (1.3 atoms of I/molecule), that, after intravenous injection into guinea pigs, the decline of specific antibody activity and radioactivity were at the same rate for ten days, and that the I^{131} activity/microgram of antibody protein was unchanged through the fifth day (see below for discussion of the effect of antibody production, Phase C). Studies of the excretion of I^{131} were consistent with the disappearance rates from the blood.

These findings were confirmed in rabbits by Dixon, Bukantz & Dammin (9) for the case of I^{131}-labelled bovine gamma globulin, and by Gitlin *et al.* (10) and Sterling (11) for I^{131}-labelled bovine gamma globulin and human serum albumin (with minor differences). Further, Dixon *et al.* reported that the I^{131} recovered in the urine was antigenically inactive, not protein-bound, and consisted of iodide and organically-bound iodine, probably diiodotyrisine. Williams [cited by Knox & Endicott (6)] showed that this compound

was diiodotyrosine (no data given). Later, Laws (12) studied the breakdown-products in the urine more extensively, and found that the I^{131} excreted was easily dialyzable. By paper chromatography, he found activity in several different spots, the most constant of which could be hydrolyzed to yield glycine, leucine, lysine, arginine, valine, glutamic acid, and radioactive diiodotyrosine.

There is thus evidence that the introduction of one I^{131} atom into each molecule of a plasma protein does not appreciably alter its immunological properties, and that this label is sufficiently stable *in vivo* so that the protein molecule most probably must be degraded before it is released. When it is released it is rapidly excreted, and does not become the object of local salvage operations. Furthermore, its presence does not seem to interfere with the enzymatic processes effecting this degradation, even by slowing their rate. It is readily quantitated. It has the disadvantages that its half-life (8 days) is inconveniently short for experiments on the persistence of antigen in tissue cells, that its radiation may interfere with cell-function, and that it cannot be precisely localized to single cells or parts of them, except in the thyroid. However, it is the best-studied, and from many points of view the most satisfactory label so far utilized in immunological studies.

Azoproteins.—The azo link has been extensively used to introduce markers into protein molecules in order to follow their fate after injection. This general approach was originated by Haurowitz & Breinl in 1932 (13) when they coupled horse serum with atoxyl (*p*-amino-benzene-arsonic acid) and traced its fate after injection by analysis of organs for arsenic. As other labelling materials have become available they have been similarly employed. Proteins colored by coupling with azo-dyes allow microscopic visualization, and radioactive isotopes introduced by the diazo reaction allow quantitation and radioautography.

Because of discrepancies in the reported behavior of I^{131}-labelled and azo-labelled proteins, Dixon, Bukantz, & Dammin (9) made a comparative study of the fate of I^{131}-bovine gamma globulin and I^{131}-bovine gamma globulin coupled with R-salt-azo-benzidine or with *p*-aminobenzoic acid [Dixon and co-workers (14)]. As determined by radioactivity, the azoproteins rapidly left the blood stream, less than 0.1 per cent remaining 24 hr. after intravenous injection; they were concentrated in the tissues (see below) and remained much longer than the lightly iodinated protein. Similar findings were independently reported by Gitlin, *et al.* (10), who also found that their azoproteins were nonhomogenous immunochemically and in the ultracentrifuge. Ingraham (1) prepared S^{35}-sulfanilyl-azo-bovine gamma-globulin containing an estimated 37 molecules and 60 molecules respectively of marking compound per protein molecule. Studying these conjugates *in vitro*, he found that 50 per cent of the label split off the protein during dialysis in 110 days.

It is apparent that azoproteins are quite different in their *in vivo* behavior from native or lightly iodinated proteins (9, 14), that the azo-linkage is somewhat unstable *in vitro* and presumably *in vivo*, and that azo-proteins

are less susceptible than native proteins to enzymatic degradation. They are, however, antigens, and they can be visualized in cells and parts of cells.

Antigens marked by the metabolic incorporation of N^{15}, C^{14}, and P^{32}.—These will be mentioned in the later discussion. Such markers, although useful for some short-term experiments, suffer from the fundamental defect that they are not metabolically inert. On the other hand, they have the fundamental advantage that the labelled material is as little altered from its native state as is conceivably consistent with marking at all. No cellular localization is possible with stable isotopes. [For a discussion of the use of isotopes in immunology, with references, see Wormall (15)].

Histochemical methods for tracing antigens.—Another approach to the tracing of immunologically active materials *in vivo* has been developed which depends on immunological activity as the marker. Marrack (16) first showed conclusively that antibodies could be coupled with an azo-dye without destroying their capacity to react with their corresponding antigen, and we have seen above that the introduction of small amounts of I^{131} did not interfere with their activity. Similarly, fluorescein was intrdouced by means of the carbamido linkage into antibody molecules (2 groups/molecule) [Coons *et al.* (17, 18]. Such solutions, when applied to antigen-containing sections of tissues, deposited precipitates over the areas containing the antigen, and these precipitates were visualized under the fluorescence microscope as bright yellow-green areas. This method detects antigen or antigenic fragments large enough to precipitate the antibody, or, if a small molecular fragment were fixed in space, large enough to react. The minimum concentration detectable is not known, although a single pneumococcus is easily visualized by this means.

The disadvantage of this method is that it has not yet been made (and would be difficult to make) quantitative. Its advantage is that it permits cellular localization of unaltered (i.e., unlabelled) antigenic material, and that it is more sensitive than are labels depending on color.

The Disappearance of Proteins from the Circulation

After the intravenous injection of a homologous plasma protein, approximately half of it disappears within 24 hr. [Glenny & Hopkins (19), Heidelberger *et al.* (20), Fine & Seligman (4), Sterling (11), Fink *et al.* (21), Miller *et al.* (22), Yuile *et al.* (23), Germuth *et al.* (24)]. It is generally agreed that this represents equilibration with extra-vascular plasma proteins. Thereafter, there is a steady decline in the level in the circulation which Glenny & Hopkins suggested was attributable to the elimination of the protein by some method of metabolism, and which Gros *et al.* (25) and Dixon *et al.* (26), have described in terms of "half-life." The latter have determined the half-life of homologous gamma globulin in seven species by injecting globulin prepared by alcohol or ammonium sulfate fractionation and labelled with I^{131}, and have compared their results with data from the literature obtained by measurements of antibody following passive immunization. The results

were in good agreement. The decay-rates were inversely related to the size of the animal and it was suggested they might be correlated with the metabolic rate. For the rabbit, the half-life of homologous globulin was 4.6 ± 0.8 days, for children (from six months to eight years of age) 20.3 ± 4.2 days, and for adults 13.1 ± 2.8 days.

Foreign proteins, at least foreign proteins derived from mammalian plasma, behave in the same way until the onset of antibody production, when they rapidly disappear from the blood stream. This was first pointed out by Glenny & Hopkins in their classical studies on the administration of diphtheria antitoxin to various animals; one can do no better than to quote Glenny's summary (27), published in 1931:

> . . . The rate of loss of heterologous antitoxin can be divided into three phases, varying in their duration with the condition of the animal in relation to the serum injected.
>
> *In normal animals. Phase A.* An initial fall of 50 per cent in antitoxic content of the blood occurring within 24 hours and often within 6 to 8 hours; the suggested explanation is that this fall is due to antitoxin distributed from the blood to the tissues. . . .
>
> *Phase B.* A gradual constant percentage loss of approximately 25 per cent from day to day, lasting usually from 6 to 7 days. This phase of gradual loss appears to be the elimination of foreign protein by some method of metabolism.
>
> *Phase C.* A rapidly accelerated loss of 50 per cent or more per day due to the active production of precipitin as a response to the injection of foreign protein. Longcope and Rackemann (1918) [(28)] showed that when horse serum is injected into a man there is a sudden loss of antigen (proteins) corresponding to a rapid production of precipitin. It sometimes happens that the amount of precipitin formed is insufficient to eliminate all the protein injected, and Phase C is followed by a gradual loss corresponding again to Phase B.
>
> *In sensitized animals* (i.e., those potentially immune to serum protein).
>
> *Phase A.* A 50 per cent fall as in normal animals.
>
> *Phase B.* A gradual loss at the same rate but of shorter duration than that of normal animals.
>
> *Phase C.* Extremely rapid elimination resulting in almost total loss in 24 hours.
>
> When homologous serum is injected, phase A loss occurs, and the rate of loss corresponding to phase B is much slower, about 20 to 25 per cent. per week, continuing until available methods of testing can no longer detect antitoxin.
>
> In animals immune to serum protein phase A is entirely masked by a very rapid elimination due to the presence of circulating precipitin.

These conclusions, obtained by bioassay of antitoxic activity, have been confirmed and extended by the use of labelled reagents. Figure 1, taken from Knox & Endicott (6), clearly illustrates the three phases described above after the administration to rabbits of from 30 to 75 mg. of bovine serum albumin/kg. These curves are identical for the first six days (phases A and B). The deviation during phase C is consistent with the presence of circulating antigen-antibody complexes detectable by radioactivity measurements but not reactive with further antibody. However, inspection of the data in

Table I, from which the upper curve was derived, indicates that this difference should not be given too much weight because of the marked variation from animal to animal in this small series. The authors point out that the time of occurrence of the "increasingly negative slope" (phase C) is "the most variable feature of antigen disappearance."

The most detailed study of phase C so far available was carried out by Talmage *et al.* (29). They measured the disappearance rate of iodinated bo-

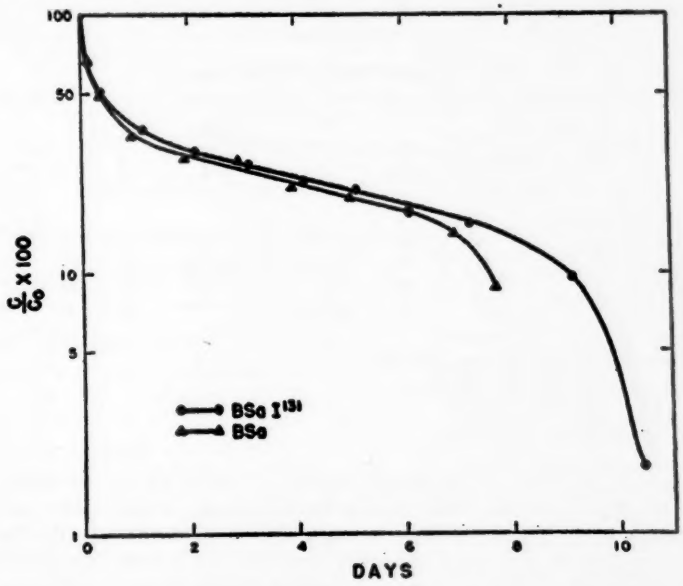


FIG. 1. Comparison of serum clearance curves of nonimmune rabbits following intravenously administered BSa and BSaI^{131}. [From Knox & Endicott (6)].

vine gamma globulin (about 0.6 atoms I^{131} molecule) after the intravenous injection of 75 mg. by radioactivity measurements (beta counts) of dried plasma. To express that percentage of antigen present at the beginning of any period which was lost in the subsequent 24 hr., ADR^2 were calculated. Antigen loss between the second and fourth days after injection was at the rate of 30 ± 5 per cent per day in normal animals for this antigen. The highest value in the group for this period was 43 per cent per day. Since this was more than twice the Standard Deviation, it was adopted as the upper limit of the decay rate attributable to nonimmune catabolism (phase B) and rates above this were tentatively assumed to be attributable to antibody. The general results of these studies are summarized in Table II. In these groups

of animals "sensitized" means with circulating antibody present (see below), and corresponds to Glenny's "immune"; whereas "anamnestic" corresponds to Glenny's "sensitized." The groups "200 r" and "500 r" were given those doses of whole body radiation 40 hr. before the administration of the protein.

The rapid removal of antigen in animals with circulating antibody, the three phases in the normal animals, and the early onset of rapid removal in animals with previous experience with the antigen are well-illustrated. The

TABLE II

MEAN ANTIGEN DISAPPEARANCE RATES*

Group	Number Animals	Days					
		0–2	2–4	4–5	4–7	7–9	9–11
Sensitized.....	5	*99*					
Anamnestic.....	8	52	*86*±16				
Control.....	21	57	31±5	*56*±15†	*79*		
200 r.....	8	57	33±5		42	*66*	
500 r.....	11	56	26±4		27	31	36

Immune ADR values are italicized.
± represents Standard deviation.
* From Talmage *et al.* (29).
† Measured on 10 animals.

suppressive effect of x-radiation on phase C removal is also clearly demonstrated. Figure 2 relates these rates to the appearance of detectable excess antibody in the plasma. Since excess antibody appeared at or shortly after the complete disappearance of antigen from the blood, and since passive administration of antibody caused a prompt disappearance of circulating antigen, phase C is clearly the result of the synthesis of antibody. Talmage *et al.* (29) also found a few rabbits in which no antibody was detectable following a phase C removal rate; these animals however had an "anamnestic" type of response to a subsequent dose of antigen 40 days later. They therefore concluded that the antigen-diasppearance rate was a rapid and sensitive method for the determination of the immune response.

These conclusions are not, however, generally applicable in detail, the disappearance rates depending on the species of animal, the dose of foreign protein, and the protein itself, For example, the onset of phase C after comparable doses of bovine serum albumin given to rabbits illustrated in Figure 1 is about two days later than that for bovine gamma globulin. No phase C is apparent in the data of Melcher *et al.* (8), who administered labelled immune rabbit globulin (iodinated anti-human serum globulin rabbit globulin) to guinea pigs, and followed its disappearance rate for ten days. How-

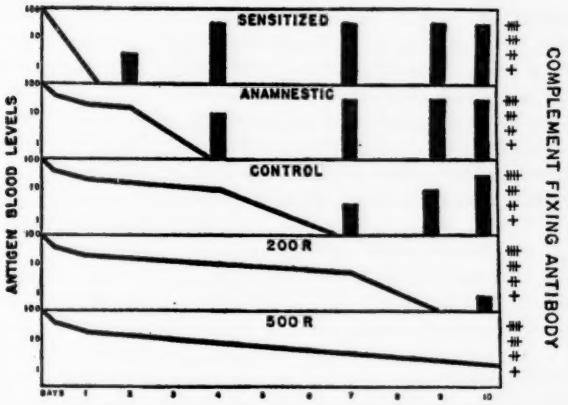


FIG. 2. Antigen elimination and antibody response. Semilogarithmic plot of mean antigen elimination from the blood (heavy line) and appearance of complement fixing antibody (solid block) after intravenous injection of 75 mg. I-BGG into sensitized, anamnestic, control, and x-rayed rabbits (groups 1 to 5). [From Talmage *et al.* (29)].

ever, they demonstrated the presence of antibody as a circulating antigen-antibody complex by the facts that (*a*) when specific antigen was added to the harvested guinea pig serum to precipitate the injected rabbit antibody, the ratio I^{131}/AbN fell from 1.4 to 0.8 between the fifth and seventh days, and (*b*) antibody against rabbit globulin could be demonstrated in the serum on the seventh and tenth days. The change in the ratio could therefore be ascribed to the presence of guinea pig antibody clinging to the precipitated rabbit globulin. With large doses of foreign protein the appearance of phase C may be greatly delayed, as in the experiments of Hawn & Janeway (30), who found bovine serum albumin circulating in the serum of rabbits injected four weeks before with a dose of 1 gm./kg. Similarly, phase C is not apparent in most of the curves of Gitlin *et al.* (10) with comparable doses of human serum albumin labelled with I^{131}, Evans Blue, Orange G, or acridine, or in those of Sterling (11) for human serum albumin, all in the rabbit. By contrast, bovine gamma globulin disappeared after the first week, even after such large doses administered to rabbits (30).

Preliminary evidence has been presented that massive dosage of rabbit with bovine serum albumin (500 mg. daily 6 days a week for from 6 to 12 weeks) resulted in "immunological unresponsiveness," so that even after the injections were discontinued, degradation continued at a slow rate for 6 weeks. No serum disease resulted. However, it is of interest that when large doses of human plasma were administered, no antibody response occurred to the major components, albumin and globulin, but serum disease nonetheless resulted, apparently from antibody formed against minor components [Dixon & Maurer (31)].

It is abundantly clear that when lightly-labelled proteins are injected, an antibody response results which leads to the rapid destruction of the bulk if not all of the antigen (see below), and that diiodotyrosine-containing peptides appear in the urine; further that the rate of excretion of split products parallels the disappearance of the intact labelled material from the blood stream. These facts indicate that after union with antibody, or indeed as a result of such union, protein antigens are hydrolyzed by some proteolytic process. This suggests that the antibody protein might also be caught in the destructive process and dismantled unless it were itself the destructive agent.

Two independent studies of this problem have recently appeared. Masouredis, Melcher, & Shimkin (32) injected 0.78 mg. antiovalbumin rabbit globulin N labelled with 0.3 atoms of I^{131}/protein molecule into each of 23 mice. Twenty-three hours later one group was given 26 μg. ovalbumin N, another group 60 μg. (both intraperitoneally), while a third group served as controls. A careful study of the subsequent events in these mice revealed that there was a prompt 8 per cent fall in the level of radioactivity in the plasma, a prompt rise in the rate of radioactivity excreted, and a temporary rise in the tissue-plasma ratio of activity in the liver and spleen, but not in the kidney. The drop in plasma activity occurred within 4 hr.; thereafter the decay curve reverted to the preinjection rate. Within 48 hr. after the injection of antigen, the mice excreted from 16 to 20 per cent more activity (derived from labelled antibody) than did the controls, and the authors pointed out that the globulin preparation administered contained from 17 to 20 per cent specifically precipitable N, suggesting that the antigen injection had brought about a complete destruction of the passively administered antibody. Complementary experiments by Melcher *et al.* (33) using labelled antigen followed by antibody in mice led them to conclude that "an *in vivo* antigen-antibody reaction is manifested by changes in plasma, excreta, and tissueI^{131} levels that are identical irrespective of the component carrying the I^{131} label."

Similar experiments in rabbits with homologous labelled antibovine serum albumin by Dixon & Maurer (34) yielded similar results; prompt removal of antigen-antibody complexes from the blood, and virtually complete destruction in some extravascular site within 48 hr.

The Cellular Deposition of Antigens

Azoproteins and the use of color.—There have been several careful studies of the cellular uptake of colored proteins, the first of which was that of Sabin (35), who injected rabbits with small but usually repeated doses of R-salt azo-benzidine-azo-ovalbumin precipitated with alum. This material was provided by Heidelberger (36), and was injected in doses of 1 to 10 mg. by the intradermal, intraperitoneal, subcutaneous, and intravenous routes, usually repeatedly (largest total dose 96 mg.) She found red dye granules most prominent in the macrophages in connective tissue and spleen, in the Kupffer cells of the liver, the lymphatic endothelium of the lymph nodes, the adventitial cells lining the sinuses in the bone marrow, in monocytes and poly-

morphonuclear neutriphiles, in fibroblasts, and adsorbed to "fibers." She noted that the amount of residual dye-protein in the liver and spleen was inversely proportional to the antibody titer in the serum. Of the cells containing dye-protein, the macrophages contained the most, the fibroblasts the least. In all these cells, the material was in the cytoplasm.

These findings have been extended by Smetana (37), Kruse & McMaster (38, 41), Gitlin (39), Latta, Gitlin, & Janeway (40), and Robertson (42). Latta *et al.* injected doses ranging from 450 to 750 mg./kg. into rabbits and sacrificed the animals at intervals up to ten days. The dye was found on microscopic examination primarily in the macrophage system throughout the body, and also in lesser amounts in the epithelial cells of the liver, the adrenal cortex, and the proximal convoluted tubules of the kidney, with traces in fibroblasts and cells of the synovial membrane. Anitschkow "myocytes" contained granules of azoprotein. Differences were noted in the distribution of human albumin labelled with different azo-dyes; although most of them were quantitative, it was found that acridine-azo-human serum albumin localized in the renal glomeruli but was absent from the tubular epithelium, whereas just the opposite localization occurred in the cases of Evans Blue- and Orange G-azo-benzidine-azo human albumin. No azoprotein was observed in cells of the lymphocyte series. All the labelled proteins were seen in the cytoplasm (40).

Kruse & McMaster were particularly interested in the lymph nodes; they found the injected azoprotein (echt-säure-blau-azo-bovine gamma globulin) in the phagocytic cells lining the lymph node sinuses, but not in the lymph follicles. It was also found in many of the other locations noted by others above, although they are not specifically described. No color was found in the hepatic cells. In a later study, these authors became especially interested in the persistence of this azoprotein in the tissues of the mouse, and were able to find traces of color remaining in the Kupffer cells and in the mesenteric lymph nodes for as long as 120 days after the injection of about 3 mg. of azoprotein. They developed a method of reversed passive anaphylaxis in mice, based on the careful observation of the vessels of the mouse ear, as a delicate index of an antigen-antibody reaction, and used it to test the tissues of the experimental mice for antigenic activity. By this means they detected the persistence of azoprotein, and of native bovine gamma globulin, in liver and lymph nodes for periods up to 120 days. They also found that albumin disappeared more rapidly than globulin, no longer being detectable after 42 days. It is to be noted that in these experiments there was a close parallelism between the persistence of labelled and unlabelled proteins of the same kind (41).

Fluorescent Antibody and the Detection of Antigen Histochemically

Another approach to the localization of antigenic material at the cellular level was made in the laboratory of the author (17, 18). This consisted of the simple expedient of carrying out a precipitin test *in situ* on a tissue section

with fluorescein-labelled antibody and studying the sections under the fluorescence microscope. A study was made in this way of the fate of ovalbumin, bovine serum albumin, and human gamma globulin injected intravenously in 10 mg. doses into mice (0.5 gm. kg.) (43). These three proteins were found in the cytoplasm of the macrophage system, and often in the nuclei as well, in the cytoplasm and nuclei of hepatic cells, renal tubular cells, and cells of the glomerular tufts. In the spleen the phagocytic cells of the red pulp contained high concentrations in the cytoplasm, and many of the nuclei contained antigen as well. The lymphoid follicles of the spleen contained lesser amounts both in the cytoplasm and in the nuclei. Fibroblasts in the perivascular connective tissue contained moderate amounts of antigen both in the cytoplasm and in the nuclei. In the hepatic and renal tubular epithelium many cells without evidence of foreign protein in their cytoplasm showed irregular patches of it in the nuclei, and, where it could be studied by comparative staining, these intranuclear aggregations seemed to be in the nuclear sap.

There is much similarity in the information obtained by the use of azo-dyes and by the use of fluorescent antibody, but there are also some differences. The azo-dyes have invariably been described as granular, and have not been reported inside the nucleus. Moreover, whereas the azo-dyes persisted for weeks, these native proteins could not be detected longer than about one week, although of course they may still have been present in concentrations too low to be detected by fluorescent antibody.

Findings more comparable with those described for azo-proteins were reported (44, 45) for the cases of pneumococcal and Friedländer bacillus capsular polysaccharides, which were also sometimes granular, which were rarely seen within the nucleus, and which persisted for months, Further, they were regularly seen in a restricted portion of the renal tubular epithelium and in the epithelium of the adrenal cortex, like the azo-proteins (40), but unlike the three native proteins.

Recently, Schiller, Shayer & Hess (46) have studied the distribution of bovine serum albumin labelled directly with fluorescein and injected into rats in doses of 25 mg./100 gms. In many other respects the distribution of the material was similar to the above descriptions, but they were unable to find any material in the nuclei. Although such labelled albumin was only slightly altered by the labelling process as determined by measurements of its physical properties (46), one experiment carried out in the author's laboratory with injected labelled bovine albumin showed a cytoplasmic distribution of a marked granular character, quite different from the often diffuse fluorescence produced by "Staining" the antigen with labelled antibody. It may be, therefore, that there is a difference in behavior endowed by the label. In this animal (mouse), labelled antigen was found in a rare hepatic nucleus.

Gitlin, Landing & Whipple (47), in an extensive investigation of human tissue with fluorescein-labelled antibodies against human gamma globulin,

human albumin, human β-lipoprotein, β_1-metal combining globulin, and human fibrin, regularly found all these substances except fibrinogen in the nuclei of many cell-types. Indeed, they were often absent from the cytoplasm.

The question of the entrance of large molecules (or perhaps immunologically active fragments of them) into the nucleus is probably most significant at the moment as a measure of the reliance which is to be put on the cytological details made visible by means of fluorescent antibody. The writer and his colleagues have presented some evidence (43) in favor of the actual penetration of the nucleus by antigen molecules, and increasing experience has assured them that if it be a diffusion artifact it is a very regular one. More experiments are needed on the point, but what little evidence there is concerning the permeability of the nuclear membrane (48) suggests, at least for the amoeba, that if anything it is more permeable than the plasma membrane.

Radioisotopes and the Uptake of Foreign Proteins by Organs and Organ-Fractions

Numerous experiments have been carried out with the object of studying the localization and persistence of radio-labelled proteins in various organs. Although it has been clearly recognized that the most impressive collections of antigen are not necessarily connected with antibody formation, there has nevertheless been the hope that such investigations might provide some information concerning it. Unfortunately, this hope is as yet largely unfulfilled.

An early and careful study of the fate and disappearance of a particulate antigen was carried out by Libby & Madison (49). They injected mice with two intravenous doses (0.65 mg.) of tobacco mosaic disease virus 18 days apart; the second dose of virus was labelled with P^{32} by adding $Na_2HP^{32}O_4$ to the nutrient solution in which the infected tobacco seedlings were grown. At the end of 24 hr., 63 per cent of the injected dose was found in the liver, and about 3 per cent in the spleen. Thereafter there was a rapid loss of activity from the liver, with a half-life of two days, and a second slower decay in the liver beginning at about the fourteenth day, with a half-life of nine days. From a consideration of the decay rates, the rise in P^{32} in the carcass, and the fact that lipid extracts of the liver taken after the fourteenth day contained from 10 to 20 per cent of the total radioactivity, they concluded that more than 99 per cent of the injected antigen had been broken down by the sixteenth day, and that the residual activity resulted from the incorporation of the products into phosphorus-containing compounds. Meantime, the mice made a good antibody response (second injection) which reached its peak on about the seventh day, declining slowly thereafter with a half-life of 6 days. These time-relationships, they stated, "can be interpreted to indicate that antigen must be present to stimulate the formation of antibody." Since their paper appeared, it has been reported that the half-life of

antibody in the mouse is 1.9 days (26), which suggests that synthesis was somewhat more prolonged in these experiments than the authors inferred, although it does not contradict their conclusion.

These experiments with tobacco mosaic virus were extended by Erickson, Armen & Libby (50) in further experiments in which TMV antigen was identifiable in liver extracts for nine days after injection, and rod-like structures similar to the virus, and unlike anything visible in normal livers, could be found by electron microscopy for 15 days. In these mice, which had not received a prior injection of antigen and hence were making a primary response, the antibody titer passed through a peak on the fifth day, and had declined almost to zero by the twelfth day.

Studies of the tissue concentration of soluble proteins trace-labelled with iodine were carried out by Dixon *et al.* (9, 14) and Latta (51), who found no storage above that which could be accounted for by the contained blood, except perhaps slight concentration in the lymph nodes (51); but azoproteins labelled with I^{131}, as reported in an earlier section, were rapidly removed from the blood and stored in the tissues, especially those rich in phagocytes, for longer than seven days (9, 14). Ingraham, employing bovine gamma globulin heavily (37, 60 groups/molecule) diazotized to S^{35}-sulfanilio acid, found storage in the liver and spleen for longer than 200 days. However, attempts to find specific antigenic activity in liver extracts after 17 days, by co-precipitation of radio-activity by the addition of nonradioactive antigen and antibody, failed (52).

The rapid disappearance of antigen from the blood, and its appearance in the liver, spleen, and lungs (53, 54) in the presence of antibody has recently been carefully explored by Garvey & Campbell (55), who found that such localization began when the antibody concentration in the rabbit was more than 0.10 mg. antibody nitrogen per ml. of serum. They also reported that the radioactivity [hemocyanin-*p*-azo-phenyl-S^{35}-sulfonate (80 to 100 groups per 200,000 mol. wt. molecule)] concentrated in the presence of antibody could be readily washed out of the liver by perfusion, and that from the perfusate immunologically active antigen and antibody could be separated 72 hr. after injection. They confirmed the more rapid excretion of radioactivity in the urine of immune animals reported by others (9, 32, 33, 53). Laws & Wright also pointed out that the much higher concentrations of antigen found in the liver of immune animals disappeared within 24 hr., and could be accounted for by split-products in the urine (53).

In an effort to uncover cytologic details of antigen-deposition, Haurowitz, Crampton & Reller (56 to 61) carried out a series of experiments in rabbits with radio-labelled antigens, measuring the residual activity, at increasing time-intervals after injection, of fractions of liver and spleen isolated by the differential centrifugation of homogenates. In all their experiments they found appreciably higher amounts of radioactivity associated with the mitochondrial fraction than with the other fractions except in the first few minutes after injection. This finding has been substantiated in

careful experiments in mice (and two rabbits) by Fields & Libby (62); these workers were able to show clearly that the amount of radioactivity associated with the mitochondrial fraction doubled when the antigen injection was made into immunized mice, that the larger the antigen molecule the greater the association with mitochondria, and that passive administration of antibody before the administration of antigen produced the same effect as active immunization. There has naturally been a considerable amount of interesting speculation concerning the significance of the association of injected antigen with the mitochondrial fraction and its possible relation to the mechanism of antibody synthesis. Recent evidence indicates however that the liver is not a site of globulin (63, 64) or antibody synthesis (65), nor, after a single injection of antigen, is more than one cell in a hundred in the spleen (66). It seems probable that much of the antigen recovered in such liver fractions came from the Kupffer cells, and most of the mitochondria from the hepatic cells. The evidence of Libby & Fields concerning the effect of antibody and particle size on the magnitude of the antigen concentration in the mitochondrial fraction enjoins great caution before one concludes that there is any special relation between antigen and mitochondria. Furthermore, without prejudice to the role of the mitochondrion in antibody synthesis, the mitochondrial fractions so far investigated were most probably derived in large part from cells which do not take part in antibody formation.

A recent report of the cellular localization of antigen by radio-autography is that of Gavosto & Ficq. They injected mice with 10 mg. doses of C^{14}-labelled tobacco mosaic virus, made histological sections of various tissues obtained at sacrifice four days later, and examined the sections for β-tracks by painting them with a 40 μ thick photographic emulsion. They found tracks emanating from "young reticular elements", histiocytes and plasma cells in the bone marrow and spleen, and from the Kupffer cells and some hepatic cells near the portal areas in the liver. Most of the tracks originated in the cytoplasm, although some rare nuclei appeared to emit electrons (67).

Antibody Formation and the Persistence of Antigen

One of the important recurring themes throughout the investigations which have been reviewed is the length of time immunologically active groups from an injected antigen can persist *in vivo*, and hence be available as possible templates active during, and guiding the specificity of, antibody synthesis. The available data are contradictory. On one hand, there is unequivocal evidence that protein antigens are rapidly destroyed *in vivo* in the presence of antibody. On the other hand, Crampton *et al.* (58, 61) have demonstrated the persistence of radioactivity 99 days after the injection of C^{14}-anthranil-azo-ovalbumin, and 93 days after the injection of S^{35}-sulfanilyl-azo-bovine gamma globulin. Subsequently, Haurowtiz, Walter & Humm (68) examined acid hydrolysates from the liver of rabbits injected four weeks before with S^{35}-azo protein. They found only about 3 per cent of the radio-

activity in the cystine and methionine isolated from the hydrolysate, and most of the residual activity in a fraction which behaved like sulfanilyl-azotyrosine on chromatograms. This indicates that the S^{35}-atom was not displaced, although the protein may have been split. Furthermore, the findings of McMaster & Kruse, summarized above (38, 41), indicate that the antigenicity of unlabelled proteins may persist for months *in vivo;* the unconventional nature of the reaction used to demonstrate this makes confirmation desirable, however.

Countervailing evidence comes from other experiments. Stevens (69) injected rabbits intravenously with very small doses (0.25 to 0.40 mg./kg.) of trace-labelled I^{131}-bovine gamma globulin. One group of his animals had been given a dose of 500 r whole body x-radiation 40 hr. before this injection; another control group was not irradiated. Four months later, on the average, he administered another dose of antigen, and measured the rate of loss from the blood as an index of antibody formation. The irradiated animals showed a disappearance rate like that produced by a first injection, not one like the controls, which made a secondary response. He reasoned that if antigen had persisted after the recovery from x-ray damage, they should then have become sensitized and capable of making an anamnestic response to the second injection. Similar results had been reported earlier by Talmage *et al.* (29). In an extensive study of the effect of x-radiation on hemolysin-production to sheep erythrocytes, Taliaferro, Taliaferro & Janssen (70) found delayed or no antibody production in rabbits stimulated with antigen from 12 hr. to 7 days after irradiation. In 16 of these animals there was no antibody production, in the remaining 32 there was marked inhibition manifested by a peak titer of less than one-ninth that of the controls, and a marked delay in the appearance of the first detectable antibody. The average first appearance of antibody in the control (nonirradiated) animals was 3.33 days, that in the irradiated animals 12.5 days. Since full recovery from radiation damage had occurred in half of six rabbits when antigen was administered 28 days after irradiation, these data can be interpreted to indicate that the effective antigen in the sheep erythrocytes was destroyed in from two to four weeks.

The data of Talmage *et al.* (29) also indicate that injected bovine gamma globulin did not survive in an effective sensitizing dose for as long as 28 days, when the animals should have recovered from irradiation damage, because they did not make a secondary response when stimulated 40 days after the first injection (the period between the twenty-eighth and the fortieth day is, however, too short for a maximal secondary response).

Finally, a long-term study by Dixon, Maurer, & Deichmiller (71) of the primary, secondary, and "tertiary" antibody responses to bovine gamma globulin and bovine serum albumin demonstrated the continuous production of antibody for 196 days after the third injection of antigen. They pointed out that the drop in antibody production after the primary response ceased with or shortly after the disappearance of antigen, but that in the anamnestic

reactions antibody production continued for six months after the disappearance of antigen from the blood stream.

There is only one case known to the reviewer where antibody production continues for some years, and where a nonliving antigen is known to persist. This is the case of the pneumococcal polysaccharides, which have been identified immunologically for as long as a year in the mouse (44, 72), which have been isolated and identified immunologically from normal human tissues at autopsy (72), and against which antibody production continues in man for as long as 8 years following the administration of 50 μgm. [Heidelberger, DiLapi, Siegel & Walter (73)]. Moreover, some of these subjects failed to show an increase in antibody when a second injection was given, although they responded to injections of a heterologous type, indicating that they were already fully stimulated.

This approaches the heart of the dilemma, because here is a clear case where antigen persists in immunologically intact form, and where antibody production continues at or near its maximum. Does this establish the template theory? Evidently not, although it is consistent with it. It is known, for example, that these polysaccharides persist in phagocytic cells (44, 45), whence they may simply diffuse out in small quantities to act as a continuous stimulus. On the other hand, it will always be difficult to establish that the last antigenically active fragment has disappeared from the last antibody producing cell. Indeed, one would be required to go further since, unless a specific "heritable" change is brought about in some cells by a first exposure to antigen (74), it will be necessary to show that antigen persists not only during the phase of active antibody production, but also throughout the much longer period during which an anamnestic response can still be elicited.

The valuable and stimulating concept that the antigen molecule functions as a template leaves still inexplicable one of the most striking aspects of the antibody response, the anamnestic reaction, as Burnet and Fenner have emphasized (74). This review, if it has done nothing else, has indicated that the static, and as yet largely qualitative and empirical methods of the cytologist, and the brutally artificial homogeneity which the chemist must produce in order to apply to tissues the classical methods of quantitation are feeble tools with which to approach intracellular events. One may hesitantly predict that when the process of antibody formation has been described in all its particularity, if it ever is, the transfer of electrostatic complementariness from antigen molecule to antibody molecule will have been found to require many steps. One can suspect that a template function of antigen will then be seen to be an early rather than a late one.

LITERATURE CITED

1. Ingraham, J. S., *J. Infectious Diseases*, **89**, 109–16 (1951)
2. Fine, J., and Seligman, A. M., *J. Clin. Invest.*, **22**, 285–303 (1943)
3. Wormall, A., *J. Exptl. Med.*, **51**, 295–317 (1930)
4. Fine, J., and Seligman, A. M., *J. Clin. Invest.*, **23**, 720–30 (1943)
5. Eisen, H. N., and Keston, A. S., *J. Immunol.*, **63**, 71–80 (1949)
6. Knox, W. C., and Endicott, F. C., *J. Immunol.*, **65**, 523–28 (1950)
7. Masouredis, S. P., Melcher, L. R., and Koblick, D. C., *J. Immunol.*, **66**, 297–302 (1951)
8. Melcher, L. R., and Masouredis, S. P., *J. Immunol.*, **67**, 393–402 (1951)
9. Dixon, F. J., Bukantz, S. C., and Dammin, G. J., *Science*, **113**, 274–76 (1951)
10. Gitlin, D., Latta, H., Batchelor, W. H., and Janeway, C. A., *J. Immunol.*, **66**, 451–62 (1951)
11. Sterling, K., *J. Clin. Invest.*, **30**, 1228–37 (1951)
12. Laws, J. O., *Brit. J. Exptl. Pathol.* **33**, 354–58 (1952)
13. Haurowitz, F., and Breinl, F., *Hoppe-Seyler's Z. physiol. Chem.*, **205**, 259–70 (1932)
14. Dixon, F. J., Bukantz, S. C., Dammin, G. J., and Talmage, D. W., in *The Nature of the Antibody Response*, 170–82 (Pappenheimer, A. M., Jr., Ed., Columbia University Press, New York, N. Y., 227 pp., 1953)
15. Wormall, A., *Brit. Med. Bull.*, **8**, 223–29 (1952)
16. Marrack, J., *Nature*, **133**, 292–93 (1943)
17. Coons, A. H., Creech, H. J., Jones R. N., and Berliner, E., *J. Immunol.*, **45**, 159–70 (1942)
18. Coons, A. H., and Kaplan, M. H., *J. Exptl. Med.*, **91**, 1–13 (1950)
19. Glenny, A. T., and Hopkins, B. E., *J. Hyg.*, **22**, 37–51 (1923)
20. Heidelberger, M., Treffers, H. P., Schoenheimer, R., Ratner, S., and Rittenberg, D., *J. Biol. Chem.*, **144**, 555–62 (1942)
21. Fink, R. M., Enns, T., Kimball, C. P., Silberstein, H. E., Bale, W. F., Madden, S. C., and Whipple, G. H., *J. Exptl. Med.*, **80**, 455–75 (1944)
22. Miller, L. L., Bale, W. F., Yuile, C. L., Masters, R. E., Tishkoff, G. H., and Whipple, G. H., *J. Exptl. Med.*, **90**, 315–20 (1949)
23. Yuile, C. L., Lamson, B. G., Miller, L. L., and Whipple, G. H., *J. Exptl. Med.*, **93**, 539–58 (1951)
24. Germuth, F. G., Jr., Oyama, J., and Ottinger, B., *J. Exptl. Med.*, **94**, 139–70 (1951)
25. Gros, P., Dubert, J. M., Sung, S. S., Coursaget, J., and Macheboeuf, M., *Bull. soc. chim. biol.*, **34**, 37–42 (1952)
26. Dixon, F. J., Talmage, D. W., Maurer, P. H., and Deichmiller, M., *J. Exptl. Med.*, **96**, 313–18 (1952)
27. Glenny, A. T., in *A System of Bacteriology in Relation to Medicine*, **6**, 157–58 (Medical Research Council, H M Stationery Office, London, England, 538 pp., 1931)
28. Longcope, W. T., and Rackemann, F. M., *J. Exptl. Med.*, **27**, 341–58 (1918)
29. Talmage, D. W., Dixon, F. J., Bukantz, S .C., and Dammin, G. J., *J. Immunol.*, **67**, 243–56 (1951)
30. Hawn, C. V., and Janeway, C. A., *J. Exptl. Med.*, **85**, 571–90 (1947)
31. Dixon, F. J., and Maurer, P. H., *Riassunti d. Comunicazioni, VI Congr. Intern. Microbiol.* (Abstract) **I**, 461 (Roma, Italia, 1953)

32. Masouredis, S. P., Melcher, L. R., and Shimkin, M. B., *J. Immunol.*, **71**, 268–74 (1953)
33. Melcher, L. R., Masouredis, S. P., and Shimkin, M. B., *J. Immunol.*, **71**, 275–79 (1953)
34. Dixon, F. J., and Maurer, P. H., *Proc. Soc. Exptl. Biol. Med.*, **84**, 442–46 (1953)
35. Sabin, F. R., *J. Exptl. Med.*, **70**, 67–82 (1939)
36. Heidelberger, M., Kendall, F. E., and Soo Hoo, C. M., *J. Exptl. Med.*, **58**, 137–52 (1933)
37. Smetana, H., *Am. J. Pathol.*, **23**, 255–67 (1947)
38. Kruse, H., and McMaster, P. D., *J. Exptl. Med.*, **90**, 425–46 (1949)
39. Gitlin, D., *Proc. Soc. Exptl. Biol. Med.*, **74**, 138–42 (1950)
40. Latta, H., Gitlin, D., and Janeway, C. A., *Arch. Pathol.*, **51**, 260–77 (1951)
41. McMaster, P. D., and Kruse, H., *J. Exptl. Med.*, **94**, 323–346 (1951)
42. Robertson, J. S., *Australian J. Exptl. Biol. Med. Sci.*, **30**, 59–71 (1952)
43. Coons, A. H., Leduc, E. H., and Kaplan, M. H., *J. Exptl. Med.*, **93**, 173–188 (1951)
44. Kaplan, M. H., Coons, A. H., and Deane, H. W., *J. Exptl. Med.*, **91**, 15–30 (1950)
45. Hill, A. G. S., Deane, H. W., and Coons, A. H., *J. Exptl. Med.*, **92**, 35–44 (1950)
46. Schiller, A. A., Schayer, R. W., and Hess, E. L., *J. Gen. Physiol.*, **36**, 489–506 (1953)
47. Gitlin, D., Landing, B. H., and Whipple, A., *J. Exptl. Med.*, **97**, 163–76 (1953)
48. Clark, A. M., *Australian J. Exptl. Biol. Med. Sci.*, **21**, 215–20 (1943)
49. Libby, R. L., and Madison, C. R., *J. Immunol.*, **55**, 15–26 (1947)
50. Erickson, J. O., Armen, D. M., and Libby, R. L., *J. Immunol.*, **71**, 30–37 (1953)
51. Latta, H., *J. Immunol.* **66**, 635–52 (1951)
52. Ingraham, J. S., *J. Infectious Diseases*, **89**, 117–29 (1951)
53. Laws, J. O., and Wright, G. P., *Brit. J. Exptl. Pathol.*, **33**, 343–53 (1952)
54. Francis, G. E., Hawkins, J. D., and Wormall, A., *Biochem. J. (London)*, **53**[3], xx (1953) (Abstract)
55. Garvey, J. S., and Campbell, D. H., *J. Immunol.*, **72**, 131–38 (1954)
56. Crampton, C. F., and Haurowitz, F., *Science*, **112**, 300–2 (1950)
57. Haurowitz, F., and Crampton C. F., *J. Immunol.*, **68**, 73–85 (1952)
58. Crampton, C. F., Reller, H. H., and Haurowitz, F., *Proc. Soc. Exptl. Biol. Med.*, **80**, 448–51 (1952)
59. Crampton, C. F., and Haurowitz, F., *J. Immunol.*, **69**, 457–59 (1952)
60. Haurowitz, F., Crampton, C. F., and Reller, H. H., *Naunyn-Schmiedeberg's Arch. exptl. Pathol. u. Pharmakol.*, **219**, 11–16 (1953)
61. Crampton, C. F., Reller, H. H., and Haurowitz, F., *J. Immunol.*, **71**, 319–24 (1953)
62. Fields, M., and Libby, R. L., *J. Immunol.*, **69**, 581–86 (1952)
63. Miller, L. L., and Bale, W. F., *J. Exptl. Med.*, **99**, 125–32 (1954)
64. Miller, L. L., Bly, C. G., and Bale, W. F., *J. Exptl. Med.*, **99**, 133–53 (1954)
65. Coons, A. H., Leduc, E. H., and Connolly, J. M., *Studies on Antibody Production. I.* (In preparation)
66. Leduc, E. H., Coons, A. H., and Connolly, J. M., *Studies on Antibody Production. II.* (In preparation)
67. Gavosto, F., and Ficq, A., *Nature*, **172**, 406–7 (1953)
68. Haurowitz, F., Walter, H., and Humm, F. D., *Federation Proc.*, **13**, 496 (1954) (Abstract)

69. Stevens, K. M., *J. Exptl. Med.*, **97**, 247–55 (1953)
70. Taliaferro, W. H., Taliaferro, L. G., and Janssen, E. F., *J. Infectious Diseases*, **91**, 105–24 (1952)
71. Dixon, F. J., Maurer, P. H., and Deichmiller, M. P., *J. Immunol.*, **72**, 179–86 (1954)
72. Felton, L. D., *J. Immunol.*, **61**, 107–17 (1949)
73. Heidelberger, M., DiLapi, M. M., Siegel, M., and Walter, A. W., *J. Immunol.*, **65**, 535–44 (1950)
74. Burnet, F. M., and Fenner, F., *The Production of Antibodies*, 2nd ed. (The Macmillan Co., Melbourne, Australia, 142 pp., 1949)

IMMUNOLOGICAL PROPERTIES OF POLYSACCHARIDES FROM ANIMAL PARASITES[1]

By José Oliver-González

Department of Microbiology, School of Medicine, School of Tropical Medicine, University of Puerto Rico, San Juan, Puerto Rico

Immunological studies of polysaccharides from infectious agents have dealt mostly with such substances isolated from bacteria and related organisms. Studies of polysaccharides from animal parasites, helminths, and more so of protozoa have been relatively few, but nevertheless results are beginning to reveal newer aspects of the field which have a great appeal for further investigation.

Polysaccharides have been prepared from various species of animal parasites mainly to be used as test antigens for the diagnosis of parasitic diseases. However, their value as diagnostic agents as well as the role which they play in acquired immunity are not understood at present. Also recent investigations have indicated that polysaccharides may be concerned with other immunological phenomena which may be harmful to the host.

Methods of Preparation and General Characteristics

Animal parasites in general are characterized by a high carbohydrate content, which serves as an important source of energy. The parasites are able to utilize simple carbohydrates present in the host and convert them into more complex ones. Also, parasites are able to utilize their own carbohydrate content while within the host to such an extent as to remain practically free of the substance. This, however, seems to occur when the parasite encounters unfavorable conditions. Absence of carbohydrate from the parasite's environment results in a decrease of the substance in it's tissues.

Little is still known about the intermediary carbohydrate metabolism of parasites. There are variations in their biochemical characteristics which seem to vary not only within species, but also within strains of a single species. For a comprehensive review of the metabolism of parasitic helminths, the reader is referred to Beuding (3).

Methods of preparation.—In general polysaccharides have been prepared from either the dry or wet whole worm material which is suspended in water and placed in a boiling water bath for 30 min. After cooling, the material which goes into solution is precipitated with cold 90 per cent alcohol. Thus protein is coagulated by heat and discarded after centrifugation. Protein is also removed by treatment of worm extracts with trichloracetic acid or by digestion with enzymes. Campbell (4) prepared a polysaccharide from *Ascaris lumbricoides* from the pig by treating whole adult worm material with

[1] The survey of the literature pertaining to this review was completed in December, 1953.

a 0.1 per cent solution of pepsin at pH 4.5. The water insoluble residue which consisted of digested protein was discarded, and the polysaccharide was precipitated from solution with chilled 95 per cent alcohol. This method, according to Campbell, had the disadvantages of possible contamination with polysaccharide from pepsin and loss of antigenicity of the helminthic material as a result of prolonged exposure to high hydrogen ion concentration.

The polysaccharides have been purified by various methods intended to eliminate minute traces of protein. Usually, this is accomplished by dissolving the crude polysaccharide in acetate buffer at pH 4.6 and discarding the undissolved material. The solution is then adjusted to pH 8.0 by addition of 1.0 *N* sodium carbonate to the solution and the polysaccharide is reprecipitated with cold 95 per cent alcohol. Repeated precipitation at pH 8.0 and solution at pH 4.6 usually yields a protein free substance.

The polysaccharides isolated in this manner or prepared by other methods usually give negative tests for nitrogen by the Nessler procedure, and give positive Molisch tests for carbohydrates at high dilutions. They are pure white substances soluble in water and they are not affected immunologically by autoclaving for 15 min. at 15 lbs. pressure.

It is to be noted that unlike the case with bacteria, worm substance is never treated with concentrated alkali or acid in order to extract polysaccharide. Treatment with concentrated acid or alkali may result in immunological differences between the substance obtained and the substance as it exists in the organism.

Very few investigators have attempted to study in detail the chemical constituents of parasitic polysaccharides. Campbell (4) reported that the polysaccharide from *A. lumbricoides* from pig consisted largely of hexoses, possibly glucose, but was free of hexuronic acids, pentoses, ketoses, and mannose. The need of such studies of other parasitic polysaccharides is becoming more and more apparent. An understanding of the immunological relationships between these substances depends largely on the better knowledge of their chemical composition.

Polysaccharides as Immunologically Active Antigens

Polysaccharides as complete antigens.—Contrary to earlier opinions, it has been demonstrated that some polysaccharides from animal parasites are complete antigens which not only react *in vitro* with antibodies but also incite their formation. Such is the case with the polysaccharides isolated from adult *A. lumbricoides* from pig (4); from the larval form of *Trichinella spiralis* (14); from adult forms of *Schistosoma mansoni*, *Fasciola hepatica*, *Necator americanus*, and *Macrocanthorynchus herudinaceous* (18), and from the larval stages of *Echinococcus granulosus* (25 to 28). High titers of precipitins and complement-fixing antibodies have been detected in animals artificially immunized with these polysaccharides.

Elaborate studies of polysaccharides as test antigens and as immunizing

agents have been carried out by Campbell (4, 5, 7). This investigator studied the immunological specificity of polysaccharide fractions isolated from various common parasitic helminths. Rabbit antiserums were prepared against whole worm material from *A. lumbricoides* from man, *A. lumbricoides* from pig, *Toxocara canis*, and *Monezia expansa*. The antiserums were then tested for precipitins against the homologous and heterologous whole worm and polysaccharide test antigens. The specificity of the precipitation reaction was far greater with the polysaccharide than with the whole worm test antigens. The polysaccharide antigens reacted only with antiserums against the whole worm material from which it had been prepared. This specificity was observed even with the closely related ascarids from man and pig, which previous to this had been found to be immunologically indistinguishable.

The specificity of polysaccharides seems to be lost, however, when antibodies develop after artificial immunization of rabbits with the polysaccharide per se, and not the whole worm material. Rabbits injected intravenously with polysaccharides dissolved in isotonic saline develop precipitins which react with other parasitic polysaccharides as well as with those injected (17, 18). This marked cross-reactivity contrasts with the specificity observed when the serum against the whole adult worm is tested against isolated polysaccharides. Such differences in specificity are difficult to explain. It may be, as suggested by Melcher (15), that polysaccharide combined with protein in its natural state is masked by the protein and plays a minor role in the stimulation of polysaccharide antibody.

Polysaccharides as test antigens for diagnosis.—Precipitating antibodies for polysaccharide which develop as a result of infection or artificial immunization have been claimed to be of diagnostic significance. The polysaccharide prepared by Melcher & Campbell (14) from the larval form of *T. spiralis* reacted to high precipitin titers with serums from experimentally infected trichinous rabbits, and failed to react with serums from animals infected with other parasites. Polysaccharides extracted from hydatid fluid and scolices reacted specifically with serums from humans infected with *E. granulosus* (25, 27, 30, 31).

Polysaccharides from animal parasites do not seem to be adequate antigens for intradermal tests. Either no reactions are observed or else large amounts of the substance are required to induce a response (4, 10, 13, 33). It is possible that the polysaccharide may be readily adsorbed by the tissue fluid and blood plasma and thus carried away from the site of injection before any reaction may be observed. Their rapid solubility in physiological solutions as well as their capacity for combining with blood agglutinins (18) also help their rapid absorption and disappearance from the site of injection.

Investigations should be continued on the nature of the reaction between polysaccharide test antigen and serums from infected and artificially immunized hosts. Also the specificity of the reaction in relation to infections with trematode and single nematode species, and particularly of those inhabiting the intestine of man, need to be examined.

Role of Polysaccharides in Acquired Immunity

A single species of parasitic worm has been shown to have a number of antigens which are responsible for the development of different types of antibodies and which, in turn, have different immunological functions. Some antigens are involved in the stimulation of antibodies which have deleterious effects on the worms and thus have a significant role in resistance to infection. These antibodies are precipitins which react with the antigenic secretions and excretions of the parasites. Precipitate is formed around the orifices and inside the worm which retards its growth, immobilizes it, and leads to its death. For an excellent review on the mechanism of acquired immunity to parasitic worms see Taliaferro (32).

Hemagglutinins and anaphylactins can also be observed to result from immunization with antigens in the different tissues of a single worm. The antigens involved in the stimulation of precipitins are apparently not those involved in the induction of hemagglutinins and anaphylactins (19, 20). Similar studies on antigens present within a protozoan species have not been made. It is very likely, however, that a similar diversity of antigens may be encountered here as in the helminths and bacteria.

Most of the evidence to date seems to indicate that polysaccharides are not involved in the stimulation of factors or of antibodies which are harmful to the parasite. Campbell prepared a polysaccharide from the larval form of the cat tapeworm *Taenia taeniaformis*, which after partial purification still contained 0.1 per cent nitrogen (6). Artificial immunization of rats with this polysaccharide resulted in partial protection against infection manifested by the development of a small number of cysticerci in the liver (early immunity) and by death of a large number (late immunity), as compared with events in uninjected controls. In this experiment resistance to infection as a result of immunization with polysaccharide may not be attributable to polysaccharide alone, since protein was also present.

Results reported by Campbell in another series of experiments gave more conclusive definition to the ineffectivness of polysaccharides in stimulating protective antibodies (7). Rats were not rendered resistant to infection with *Cysticercus crassicolis* by previous injection of nitrogen free polysaccharides isolated from the larval material. Also the protective value of anti-cysticercus serum was not removed by treatment of the serum with polysaccharide. Furthermore, specific anti-polysaccharide serum had no passive protective effect. The effect of artificial immunization with polysaccharide on resistance to infection should be studied in relation to other parasitic species.

If polysaccharide antibodies developing either as a result of infection or of artificial immunization do not seem to be involved in promoting resistance, there is the possibility that such antibodies may have other immunological functions which could be responsible for deleterious effects on the host.

Polysaccharides and Other Immunological Phenomena

Relationship to hypersensitivity and cross-sensitization.—Polysaccharides are capable of sensitizing animals to such a degree that anaphylactic shock may be produced with a challenging dose of the same substance. The results of Campbell (4) with ascaris polysaccharide indicated that sensitization, as shown by anaphylaxis, was not attributable to contamination with protein, but was a true response to polysaccharide. Thus guinea pigs sensitized to protein or polysaccharide were more sensitive when challenged with the homologous than with the heterologous antigen. Similarly, Sprent (28) observed anaphylactic shock in guinea pigs sensitized to ascaris through infection or artificial immunization, when challenging doses of protein-free materials, mostly polysaccharide, were administered. The polysaccharides were found to contain minimal amounts of nitrogen, but as suggested by Campbell's results, sensitization to polysaccharides, as observed by Sprent, may have been independent of sensitization to proteins (4).

The sensitization to polysaccharide during infection observed by Sprent (28) suggests that the sensitizing agent is released by the parasite either when migrating through or when permanently established in the host's tissues. Furthermore, there is an apparent cross-sensitization between organisms as observed during infections with ascaris and trichina and with other parasitic nematodes (2, 12, 26, 29). The excretions and secretions of living worms involved in sensitization are polysaccharide in nature and have common immunological properties. When the larvae of *T. spiralis* and adults of *S. monsoni* were incubated in human serums for periods up to 24 hr., a reduction was observed in the polysaccharide content of the whole worm (21). Parallel to this, the living worms released into the incubating serum-medium a polysaccharide which had the same immunological effect upon the blood serum isoagglutinins as did the chemically isolated polysaccharide. The titer of the α_2 isoagglutinins were reduced to zero, thus suggesting that the secretions and excretions from the two species of parasites are immunologically related. If such related polysaccharides are released during infection by all animal parasites and if such substances are directly concerned with hypersensitivity, it may follow that during infection with a single organism the host becomes sensitive to substances from other infectious agents.

Relationship to sheep cell antigens.—Antigens related to red cell agglutinogens (i.e., Forsman antigens) are scattered among animals and plants, although more or less characteristic of certain groups or related species. They are found in the erythrocytes and tissues of certain animals and in certain strains of bacteria, as well as in animal parasites. Two of the outstanding immunological characteristics of these antigens are that they incite the formation of sheep cell lysins when injected into rabbits and that they are related to the group A human erythrocyte agglutinogen. Chemically, the Forssman antigens are lipopolysaccharide in nature.

Although reports on the presence of Forssman antigen in animal parasites have been very few there is indirect evidence suggesting that polysaccharides from parasites are related to these heterogenetic substances. Mauss (13) reported the presence of lysins for sheep erythrocytes in the serums from rabbits infected with *T. spiralis*. The relationship to the Forssman antibody was established by the fact that the hemolysin was absorbed by guinea pig kidney emulsion and that the rabbit serum produced anaphylactic-like symptoms when injected into guinea pigs and chickens. Noronha-Peres (16) reported high titers of sheep cell hemolysins in 20 individuals infected with *S. mansoni*. The titers of the hemolysins ran from 1:56 to 1:244 as compared with lower titers observed in normal individuals. Arana (1) obtained high titered hemolysins and agglutinins for sheep cells in humans previously infected with *E. granulosus* and injected subcutaneously with fluid from the hydatid cysts. These antibodies were not increased in normal individuals; previous sensitization was necessary for the increase in titer.

Further evidence for the relationship between polysaccharides from certain of the animal parasites and the Forssman antigens is the fact that anti-Forssman serums treated with the polysaccharides lose their property of lysing sheep erythrocytes. The polysaccharides from *A. lumbricoides* from pig and from other parasites inhibit the hemolysin for sheep cells found in the serum of trichinous rabbits as well as in certain antibacterial serums (18).

The ability to inhibit hemolysis of sheep cells may be a specific property of the polysaccharides from infectious agents without any relation necessarily to their properties as Forssman antigens. Also, the polysaccharides from infectious agents may be related to other antigens in the sheep erythrocyte stroma independent of the Forssman antigens.

Relationship to human red blood cell agglutinogens.—The relationship between substances in infectious agents and blood agglutinogens was originally observed with certain species of bacteria. Sera from rabbits immunized with the Shiga dysentery bacillus and with certain strains of *Salmonella schottmuelleri* were found to agglutinate human erythrocytes of groups A and AB, but not of groups O and B. It was shown later, however, that the serums from rabbits injected with dysentery bacilli reacted most intensely with human blood group O and subgroup A_2 erythrocytes, and therefore, the bacterial hemagglutinins resembled the atypical human isoagglutinin α_2, also termed anti-O (12). Agglutinins acting on human blood cells of all groups have been found to be formed following immunization of certain animals with pneumococci of type XIV. Whether agglutination of human erythrocytes of groups A and AB results from the presence of the Forssman antigen in the pneumococci, of some other common antigen, remains to be investigated.

Relationship to the A_2-agglutinogen of human erythrocytes.—The relationship between polysaccharides from animal parasites and human blood cell agglutinogens was first established by the fact that polysaccharides extracted from *A. lumbricoides* from pig, when added in suitable concentrations to

human serums of blood groups B and O, caused a marked diminution of the α_2 isoagglutinin titer of the serum (18). In most instances the titer of this agglutinin was reduced to zero. There was also a less marked reduction of the α_1 titer, but this may have been attributable to the fact that the α_1 and α_2 agglutinins are immunologically closely related and bound together. The polysaccharides from other parasitic worms, such as the adult forms of *S. mansoni*, *Taenia saginata*, *F. hepatica*, *N. americanus*, and the muscle stage of *T. spiralis* had a similar effect on the α_2 isoagglutinin content of serums (22). Polysaccharides injected intravenously into monkeys and rabbits also caused an inhibition of the α_2 isoagglutinins in the living animal (23). Since there is an apparent specificity for the α_2 isoagglutinins, the polysaccharide has been termed the A_2-isoagglutinogen-like substance of animal parasites. If polysaccharides inhibiting isoagglutinins are present in the tissues of the parasites or exist as metabolic products, it would be of great interest to determine whether during the infection the host becomes immunized to these substances with a resulting effect on the isoagglutinin titers in the host's serum. Such changes might, in turn, be part of a chain of events resulting in autoagglutination of the host's erythrocytes.

Although no attempts have ever been made to isolate the A_2 isoagglutinogen-like substance from malarial parasites, there is some indirect evidence to show that there exists some factor within the parasite which alters the isoagglutinin content of the host's serum. The serums from patients suffering from repeated attacks of malaria showed increased α and β agglutinin titers as compared with serums from patients after a single attack or from normal individuals (18). The increase in isoagglutinin titer is apparently related to the degree of antigenic stimulation, since the highest titers were observed in individuals who had suffered the largest numbers of malarial attacks. Two patients with clinical manifestations of blackwater fever also had markedly increased α and β agglutinin titers (22). No differentiation was made in these cases between the α_1 and α_2 antibodies. Since blackwater fever is associated with infection with malarial parasites, this adds more evidence to the suggestion that some factor in the organism may be responsible for the increase in isogglutinin titers. Whether this factor is a polysaccharide, as seems to be the case with parasitic helminths, remains to be seen, since polysaccharides have not yet been isolated from any of the parasitic protozoans.

Autoagglutinins and agglutinated red blood cells have been also observed in the blood of individuals with circulating microfilaria of *Wuchereria bancrofti* (11). Since the isoagglutinogen-like substance has been found in various helminths, it is logical to assume that such a substance may be also present in *W. bancrofti*.

It may be a physiological function of infectious agents to secrete polysaccharides having blood agglutininogen properties. It also may be a function of the normal blood isoagglutinins to bind these substances by combining with them, but in instances of repeated infection or multiplication of para-

sites, there may be an excess of polysaccharide which possibly immunizes the host, leading to the development of antibodies which might agglutinate the host's own erythrocytes.

Another finding to be considered is that polysaccharides extracted from animal parasites can be adsorbed onto human red cells of Groups O and B (24). The erythrocytes thus acquire characteristics of Group A_2 erythrocytes (this would be the case for erythrocytes of Groups A_1, B, and O), with the ability to inhibit the α_2 agglutinins in Groups O and B serums. With this change in antigenicity there are two related phenomena which could be responsible for autoagglutination of erythrocytes: (*a*) Anti-polysaccharide or isoagglutinin-like antibodies develop as a result of immunization of the host with the substance released by the infectious agent, and hemagglutination follows, depending on the individual's own blood group. (*b*) Polysaccharides or isoagglutinogen-like substances are adsorbed onto red cells, giving A_2 characteristics to the cells. The red cell-polysaccharide complex may be agglutinated by natural or incited agglutinins in circulating plasma. Each process may work independently, but it is very likely that they are both closely related.

Relationship to cold agglutinins.—Agglutinins acting at 6°C. have also been detected in serums from humans infected with animal parasites. The serums from 24 out of 26 patients with recurrent infections with malarial parasites, and in 12 out of 14 patients with *Leishmania donovani*, revealed high-titered cold agglutinins (8, 9). Cold agglutinins were also observed in the serums from two cases of blackwater fever (22).

For complete absorption of cold agglutinins the absorbing material and serum should be kept at 6°C. for prolonged periods of time. Treatment of serums at room temperature or at 37°C. results only in partial absorption. Polysaccharides from *A. lumbricoides* from pig, and the larval form of *T. spiralis* inhibited to completion the agglutinins acting at 6°C. present in normal Group O and B human serums when absorption was carried on at 6°C. (23). Although cold agglutinins may be absorbed by a variety of substances, such as erythrocytes of any blood group and by human and animal tissues, such absorption may be related to the presence of specific polysaccharides in the absorbing materials. The relationship known to exist between the α_2 and cold agglutinins also suggests that a common antigenic substance is responsible for the development of both antibodies.

Summary

Investigations carried out up to now with polysaccharides from animal parasites are very meager, and in some instances have revealed contradictory findings which require further clarification. Among the various problems, one of the most important to be studied is whether polysaccharides are actually species-specific, or whether they represent an antigen common to all parasitic species. Thus far, various immunological properties have been de-

scribed which are mutually possessed by the polysaccharides. These may be summarized as follows: (*a*) The substance, as isolated from whole worm material inhibits the α_2 isoagglutinins in human serums. It also inhibits the hemolysins for sheep cells in serums from infected animals. (*b*) Inhibition of the α_2 agglutinins is also accomplished in the living animal after the intravenous injection of the polysaccharide. (*c*) Injection of polysaccharides from various species of parasites into rabbits results in antiserums which cross-react to high titers with the heterologous polysaccharides. (*d*) Polysaccharides can be adsorbed onto human red blood cells of Groups O and B, which in turn, inhibit the α_2 agglutinins in human serums. These common immunological properties characterize the polysaccharides not as species specific, but as heterogenic antigens. Thus, their value as diagnostic agents is very questionable.

There is some evidence indicating that polysaccharides may be harmful to the host. They may be associated with hypersensitivity. Also, if secreted by the organisms during actual infection, they may adsorb onto the erythrocytes leading to the formation of erythrocyte-polysaccharide complexes, foreign to the host, which may incite the formation of autoantibodies and thus lead to agglutination and hemolysis of the host's erythrocytes.

The findings thus reported certainly indicate that further studies of polysaccharides might reveal many other interesting immunological phenomena.

LITERATURE CITED

1. Arana, A. J., *J. Immunol.*, **48,** (3), 203–11 (1946)
2. Bakon, B., and Brunner, M., *J. Allergy*, **13,** 459–66 (1942)
3. Beuding, E., *Physiol. Revs.*, **29,** 195–218 (1949)
4. Campbell, D. E., *J. Infectious Diseases*, **59,** 266–80 (1936)
5. Campbell, D. H., *J. Parasitology*, **23**(4), 248–53 (1937)
6. Campbell, D. H., *J. Immunol.*, **35,** 195 (1938)
7. Campbell, D. H., *J. Infectious Diseases*, **65,** 12–15 (1939)
8. Conte-Marotta, R., and Tropeano, L., *Boll. soc. ital. biol. sper.*, **22,** (12), 41–48, (1946)
9. Conte-Marotta, R., *Boll. soc. ital. biol. sper.*, **22,** (2) 67–69 (1916)
10. Feinberg, J. G., and Morgan, W. T., *Brit. J. Exptl. Pathol.*, **34**(1), 104–18 (1953)
11. Franks, M. B., *Proc. Soc. Exptl. Biol. Med.*, **62,** 17 (1946),
12. Landsteiner, K., and Levine, P., *Proc. Soc. Biol. Med.*, **28,** 309–35 (1930)
13. Mauss, E. A., *J. Immunol.*, **42,** 71–77 (1941)
14. Melcher, L., and Campbell, D. H., *Science*, **96,** 431–32 (1942)
15. Melcher, L., *J. Infectious Diseases*, **73,** 31–39 (1943),
16. Noronha-Peres, J., *Rev. brasil biol.*, **4**(3) 401–4 (1944)
17. Oliver-Gonzalez, J., *J. Parasitol. Suppl.*, **30,** 12 (1944),
18. Oliver-González, J., *J. Infectious Diseases*, **74,** 173–77 (1944)
19. Oliver-González, J., *J. Infectious Diseases*, **78,** 232–37 (1946)
20. Oliver-González, J., *J. Infectious Diseases*, **85,** 66–71 (1949)
21. Oliver-González, J., *J. Infectious Diseases*, **79,** 221–25 (1946)
22. Oliver-González, J., *Proc. Sod. Exptl. Biol. Med.*, **57,** 25–26 (1944)

23. Oliver-González, J., *Proc. Soc. Exptl. Biol. Med.*, **82**, 559–61 (1953)
24. Oliver-González, J., *Proc. Soc. Exptl. Biol. Med.*, **84**, 520–22 (1953)
25. Pirosky, I. R., and Frocheschi, C., *Rev. inst. bacteriol. "Carlos G. Walbran,"* **10**, (2) 230–32 (1941)
26. Schonfeld, W., *Arch. Dermatol. u. Syphilis*, **175**, 54–90 (1937),
27. Senekji, H. A., *Trans. Royal Soc. Trop. Med. and Hyg.*, **34**(15), 401–3 (1941)
28. Sprent, F. J., *J. Infectious Diseases*, **84**, 221–29 (1949)
29. Sprent, F. J., *J. Infectious Diseases*, **86**, 146–58 (1950)
30. Szaflerski, J., and Nawrocki, J., *Med. Weterynar.* (*Poland*), **7**(1), 16–19 (1951)
31. Tagle, V. I., *Bol. inform. parasitol. Chilenos*, **6**(3), 36–37 (1952)
32. Taliaferro, W. H., *Physiol. Revs.*, **20**(4) 469–92 (1949)
33. Wharton, D. R., Cuervo, C., and Moyer, V. A., *J. Infectious Diseases*, **51**, 254–64 (1947)

NONSPECIFIC FACTORS IN IMMUNITY[1,2]

By W. J. Nungester

Department of Bacteriology, University of Michigan, Ann Arbor, Michigan

The problem of resistance to disease is complicated not only by the variety of mechanisms involved, but also by the variety of agents to which the host is naturally resistant or susceptible. In the infectious diseases these agents may be either microorganisms (bacteria, rickettsiae, viruses, protozoa, or fungi) or the toxins produced by such organisms. In the allergic diseases the agents are a wide variety of allergens to which some people are highly susceptible, others not at all. And, although in the neoplastic diseases the etiologic agents are not nearly so well understood, there is mounting evidence that these afflictions, too, depend on the lowered resistance of the host to the disease agent. The study of immunology with respect to tumors has long been neglected, but current experimental approaches clearly suggest a fertile field for investigators who want a better understanding of the well-recognized differences in natural susceptibility of the individual man or animal to cancer.

Broad as are the manifestations of host resistance in markedly different types of disease, one cannot avoid the thought that underlying these apparently unrelated natural defenses are common mechanisms. If we had a clear and fundamental understanding of why a given host possesses a natural resistance to one disease and not another, the general approach of applying such knowledge to many diseases would not be too difficult. It is not yet clear why one species of animal resists the invasion of a given microorganism, while a phylogenetically related species is highly susceptible to the same pathogen. Here is a challenging area for fundamental and imaginative research.

It is safe to anticipate that in investigations yet to be made more attention will be directed to reducing to a biochemical basis the conflict between host and parasite. A pneumococcus, for example, is not virulent because it has a capsule, but rather because its capsule contains certain chemical substances which react with counter biochemical systems in the host in one way or another, depending on whether or not the host is susceptible to this pathogen. The adult host may resist invasion by a parasite not only because of previous exposure to subinfective doses of the organism with resulting active immunization but also because of changes in biochemistry which occur in the normal development of the individual.

Before reviewing current advances in our knowledge of the mechanisms of natural or innate host resistance, it may be helpful to summarize the

[1] The survey of literature pertaining to this review was completed in February, 1954.

[2] The following abbreviations have been used in this chapter: PAB (*p*-aminobenzoic acid); L.P.F. (leukocyte-promoting factor).

general factors which determine the resistance of an animal to infectious diseases. The phenomenon of species specificity in host resistance occurs over and over again, and for this reason it is impossible to estimate the resistance of a given species to a given parasite on the basis of data obtained in studies of a different species. The age of the host also is important in determining resistance. In some instances the very young are most susceptible; in others, the mature individuals.

The nutritional state of the host may lessen or increase his resistance to infection (1, 2, 3). The viruses which depend on the metabolism of the host cells for their proliferation may find it difficult to grow in the cells of an undernourished host (4). The presence of disease, infectious or noninfectious, may decrease or increase the resistance of the host to the invasion of pathogenic microorganisms. The patient suffering from cardiac decompensation is particularly susceptible to pneumonia, and the diabetic patient to pyoderma. On the other hand, evidence has been collected, especially in animal experimentation, which shows that infection with one pathogen may interfere with invasion by another. Although most studies of this type involve viruses (5 to 9), interference has been observed in mixed infections with the helminths (10).

Currently, we are aware of the role of anatomical structure in defending the host against the invasion of microorganisms. The covering epithelium, and the fascia in the deeper body structures, have been studied by anatomists and surgeons in terms of the ability of such barriers to prevent the invasion or spread of microorganisms. We know what some of the physiological defense mechanisms are: for example, the flow of tears washes away bacteria from the conjunctiva, ciliary action moves the nasal, tracheal, and bronchial mucus with trapped microorganisms (11), and forceful expiration, as in coughing, raises excessive quantities of mucus from the lower respiratory tract (12). The many current studies on the effect of endocrine preparations on host resistance have recently been reviewed (13), and in this study it was pointed out that ACTH and cortisone were effective in the treatment of diseases characterized by a deposition of fibrin, as in rheumatic disease, but that in other infections these drugs actually lowered resistance. Several ways in which these hormone preparations could lower resistance were suggested: inhibition of the inflammatory reaction by decreasing capillary permeability, with a resulting decrease of blood serum proteins in and around the infected tissue; decrease of infiltration of phagocytic cells; inhibition of antibody production; and a possible decrease in activity of the fixed phagocytic cells of the reticuloendothelial system. Since this review was published its general conclusions have been supported by additional information. The suppression of granuloma formation by cortisone has again been reported (14), and the resistance-lowering effects of cortisone in virus infections (15) and bacterial infections (16, 17) have been observed. Further studies of the effect of cortisone on antibody production have led to varying results. For example, it has been claimed (18) that in rabbits simultaneously immunized

with bovine serum albumin and sheep erythrocytes, cortisone depressed the production of antibodies to the serum albumin but had little or no effect on the formation of antibodies to the red blood cells. We must confess ignorance of the underlying mechanisms of antibody production, despite much speculation. Cortisone has recently been shown (19) definitely to depress the ability of the reticuloendothelial cells of the rat to remove bacteria from the blood. The clinical observation that patients with Cushing's syndrome, in which there is hypoactivity of the pituitary gland, are unusually susceptible to upper respiratory tract infections also suggests a role of the endocrine system in host resistance.

Both the anatomy and physiology of the respiratory tract must be considered in understanding the role of particle size in determining whether or not an air-borne particle contaminated with microorganisms will reach the alveoli of the lungs and be retained. This has been recently studied in animals (20) and in man (21). Results of these studies and others indicate that only small particles, those less than 3 or 4 μ, reach the lung parenchyma, and particles smaller than 0.1 or 0.3 μ will not be retained in the alveoli.

In current investigations of host resistance, attention has been directed primarily at the humoral and phagocytic defense mechanisms. This does not mean that other mechanisms are of minor significance, but rather that we are still interested primarily in the defense systems described by the early pioneers, Nuttall, Bordet, Metchnikoff, Wright, and Douglas. Without doubt, we could broaden our research interests to great advantage in terms of gaining a more adequate understanding of host resistance. Nevertheless, real progress has been made even with attention focused only on the humoral and phagocytic defenses of the host. Much yet remains to be done on these two facets of host resistance, particularly from a comparative point of view, in explaining the resistance or susceptibility of a given host, species or individual, to a given parasite.

Humoral Defenses of the Host

Blood serum.—Blood serum is much more complex than it was believed to be even a few years ago. Immunologists studying active, and sometimes difficult to control, reactions involving antibodies and complement have gained a greater appreciation of the complexity of serum than the biochemist has. As we review the subject of serum as studied by the immunologist, the limitations of our current knowledge will be more apparent. In this review we will be concerned with normal serum rather than with serum from animals which have been purposely immunized. However, it must be borne in mind that a "normal" animal or man may have had immunological experiences beyond the control or knowledge of the investigator. Even animals raised in a germ-free environment (22) are not free from contact with chemical substances which may mimic in part the molecular configuration of some antigens found in a pathogen being studied, with the result that the serum may contain small quantities of antibodies. This is all the more true with normal

laboratory animals, which usually have had a previous experience of infection with one or another microorganism.

It is especially important to understand the role of normal serum in the host-parasite relationship, since serum or plasma is the first body defense met by the parasite once it breaks through the skin or the epithelial lining of viscera serving as the portal of entry of the pathogen. Serum components may also be found in normal secretions such as those of the respiratory tract, or in fluids accumulating in injured tissue such as the alveoli of an inflamed lung. Also, components of serum are present to a limited and varying degree as a lubricant on the various serous surfaces: peritoneum, pleura, pericardium, and the synovial membranes of joints. Any trauma in these tissues or alteration in their physiology results in a marked qualitative and quantitative change in the serum components in these parts. Small quantities of some of the constituents of serum, particularly the smaller molecular components, are also found in spinal fluid.

From the immunologist's point of view, serum contains antimicrobial components which can be inactivated within 10 min. at 55°C. and are therefore considered heat-labile, and others which remain active after such treatment and are referred to as heat-stabile factors. The heat-labile factors are particularly active against gram-negative organisms, and the heat-stabile factors may be germicidal for gram-positive organisms [Mackie & Finkelstein (23)]. Since hemolytic complement is also heat-labile, it is understandable that confusion has arisen as to the relationship between the hemolytic complement and the antimicrobial factor or bactericidal complement. Likewise, the heat stability of the serum component which acts on gram-positive bacteria and specific antibodies has caused some writers to refer to this component as a natural antibody when speaking of the antibacterial action of serum against the gram-positive organisms. A third antimicrobial property of serum is associated with the lipid fraction and has been studied particularly for its action on viruses (24, 25, 26).

Heat-labile serum component.—Serum complement has been critically studied in recent years, both for its hemolytic action on erythrocytes first treated with specific immune serum and for its nonspecific antimicrobial action against gram-negative organisms. Ecker and his colleagues are to be credited with the pioneer studies of the fractions of complement which have now been separated and studied for physical, chemical, and biological properties (27, 28, 29). A very readable review has been published by Heidelberger (30). At least four of the components of complement have been separated and characterized chemically and biologically to some degree; these are designated C′1, C′2, C′3, C′4. The C′1 fraction has been purified and characterized chemically as a euglobin. The C′2 and C′4 components have been obtained as a mucoglobulin mixture. The C′3 fraction was found to be present in lowest titer in human complement; this fraction, therefore, is the limiting component in the biological activity of serum from man. It appears to be a lipoprotein. None of the components are active alone in either the

hemolytic or bactericidal systems, but they act together to bring about hemolysis of sensitized eyrthrocytes or destruction of susceptible microorganisms.

C'1 combines with sensitized cells but alone is hemolytically inert. C'4 does not combine in the absence of C'1. C'3 is not fixed by antibody-treated erythrocytes, but it is essential for hemolysis; its action appears to be catalytic, since it causes hemolysis after fixation of C'1, C'2, and C'4 to the susceptible cell. The C'1 and C'2 components are heat-labile, while C'3 and C'4 are heat-stabile. An analysis of horse and guinea pig serum as related to conglutination reveals four similar components [Rice (31)]. The limitations of the current biochemical procedures for separation and identification affect the interpretation of these findings. Valuable as the separation of complement into four components has been, it is probable that further investigations will give still more meaningful information to aid the immunologist in understanding the special biological properties of complement, and particularly its function *in vivo*.

The earlier investigators of the bactericidal properties of serum attributable to the heat-labile or complement portion did not have available information on the fractions of complement. Hence, the generalization that gram-negative organisms were peculiarly susceptible to unheated serum did not differentiate hemolytic from bactericidal complement. However, early work of Bordet (32), cited by Tilden (33), as well as studies made more recently by others (34, 35, 36), cast doubt on the identity of hemolytic and germicidal complement. There is need to re-study this problem of the relation between hemolytic and bactericidal complement, directing attention to quantitative studies with the four currently recognized components. Such a start has been made by Seifter, Dozois & Ecker (28), but the field is yet to be adequately developed. Future investigations will be of most help in understanding host resistance if the pathogens selected for study have a known virulence for the host from which the serum is obtained. It will also be desirable to study quantitatively the effects of the components, individually or in mixtures, on graded doses of the pathogen, an approach used by Adler (37).

Besides acting on gram-negative bacteria, bactericidal complement has also been shown to have an antimicrobial action on some gram-positive bacteria (33, 38), viruses (34, 39, 40), and protozoa (41, 42). Recently, a marked *in vivo* effect of guinea pig serum on a transplantable mouse lymphoma has been described by Kidd (43). Since no such effect was noted *in vitro*, it was concluded that guinea pig serum, or more specifically, a heat-stabile component of complement, acts in conjunction with a serum component of the mouse. The difficulty of heterotransplantation of animal tumors or homografting of skin in man may possibly be related to a complement factor acting with "natural" antibodies.

Various investigators have studied complement for its effects on phagocytosis, and their results differ. There is no doubt that normal serum increases phagocytosis over that observed in saline (0.85 per cent NaCl) solutions,

hence the terms *normal opsonins* and *bacteriotropins*. However, it is less clear what role the heat-labile component plays. It is possible that the divergent opinions arise from tests with organisms having different virulence for the host from which the phagocytic cells were obtained (44, 45, 46). The possibility that complement increases phagocytosis in the presence of small amounts of specific antibody is supported by recent findings [Maurer & Talmage (47)] that complement increased the amount of precipitate in a system containing a soluble antigen and specific antiserum. This of course is directly related to the phenomenon of conglutination. The role of the several components of complement in the phenomenon of conglutination have recently been investigated [Rice (31), and Blomfield (48)]. It has also been demonstrated that factors C′2, C′3, and C′4 enhance the virucidal properties of antiserum against Western Equine Encephalomyelitis virus [Dozois & Wagner (49)].

Complement as measured by the hemolytic reaction varies from species to species, and from normal to diseased individuals. In disease a drop in complement titer may indicate a bad prognosis for the patient. Ecker, Seifter & Dozois (50) studied the change in the various components of complement in 300 patients. In the group of patients in which there was a small or no change from the normal level of the several complement components 5 per cent died. In the group in which there was a marked decrease in the C′4 and C′2 components and no significant change in the C′1 or C′3 fraction 34 per cent died.

The possible role of plasmin in bringing about such *in vivo* changes might well be investigated, since Pillemer *et al.* (51) have shown that it inactivates complement *in vitro*. Stress conditions, such as x-irradiation of the whole body, have been shown to decrease the bactericidal activity of serum [Marcus & Donaldson (52)]. On the other hand, pain stimulation (53) or stimulation of the abdominal sympathetic nerves (54) increased the antibacterial properties of serum for both gram-negative and gram-positive organisms.

Heat-stabile serum component.—The heat-stabile component in serum which in general is bactericidal for gram-positive organisms was first described by von Behring and designated as beta lysin. Its antimicrobial action varies with the species of host from which the serum was obtained and the bacterium used as the test organism. Although it is highly probable that this factor plays a role in host resistance, it is also true that host resistance to a pathogen cannot be measured solely in terms of the beta lysin activity of its blood serum. For example, Ledingham (55) found that both rat and rabbit blood were highly anthracidal, yet the rat was resistant and the rabbit susceptible to anthrax. The beta lysin factor or factors have been investigated for their action on various bacteria (56, 57, 58). Petterson (59) has demonstrated two factors responsible for this antimicrobial action in heated serum. One, which he referred to as the activating factor, was adsorbed to the susceptible bacterium, and the second was not adsorbed primarily to the organism.

It is possible that in our zeal to isolate heat-labile and heat-stabile serum factors and to study each separately for antimicrobial properties, we may be missing some of the *in vivo* phenomena which may depend on interactions between all the constituents of serum, or better, plasma. Furthermore, since animals and man are never free of previous immunological experiences, it is necessary to consider the role of "natural" antibodies as affecting the antimicrobial action of both the heat-labile and heat-stabile factors *in vivo*. Therefore, desirable as it is to isolate the various humoral factors and to study each separately, it is also in order to study blood plasma or serum *in vitro*, taking care to maintain the system as physiological as possible, including the prevention of loss of carbon dioxide with a resulting shift in pH.

In recent work, Hench (60) compared the antimicrobial action of fresh serum from guinea pigs and from rats on graded doses of *Diplococcus pneumoniae*, Type I. There was as much as a thousandfold difference between the antibacterial actions of the two types of serum. From these tests it would appear that the guinea pig is a million times as resistant to the test organism as is the rat. Some evidence was obtained indicating that the germicidal action of guinea pig serum is related to an oxidation reduction potential unfavorable to survival of the pneumococcus.

Lipid serum component.—Serum lipids have been related to virucidal properties of serum (24, 25, 26). Again the problem arises as to whether or not this effect is entirely attributable to the lipids *per se*, or whether it may involve "natural" antibodies or other serum constituents. The role of lipids in antigen-antibody reactions has been recently re-examined by Krueger & Heidelberger (61) and Orlans (62), and it would appear that lipids increase the rate of antigen-antibody reaction. Therefore, it seems reasonable at present to attribute to blood lipids some effects in host defense, as a result of either direct action on some classes of infectious agents, such as viruses, or indirect action in aiding natural or specific antibodies in their defense of the host against pathogenic microorganisms or their toxins.

Enzyme in serum.—That the nonspecific bactericidal power of serum is related to enzymes in the blood, at least for the nonpathogenic test organism *Bacillus licheniformis*, is suggested by the recent experiments of Burdon & McRoberts (63) with human serum in the acute phases of various infectious diseases. In 12 of 15 such serums showing marked bactericidal power, the proteolytic action of the serum as measured by the gelatin film technique ranged from 100 to 175, with a mean of 131, as compared to a mean of 75 for normal serum. When the serum was treated to remove its proteolytic activity, the bactericidal properties disappeared.

Other Body Fluids

Spinal fluid.—Normal spinal fluid has not as yet been demonstrated to contain bactericidal or virucidal factors. The diffusion of proteins from spinal fluid to blood is marked. Antibody solutions injected intrathecally rapidly pass from the spinal fluid to the blood. Such solutions injected intravenously

do not reach the spinal fluid in any but minimal quantities, even in the presence of meningitis. Hence it is not surprising to find a marked difference in the actions of these two body fluids on microorganisms. It must be stated, however, that investigations on the antimicrobial action of spinal fluid have been limited. Lysozyme has been demonstrated in the spinal fluid of children (64). Although lysozyme is very potent in destroying and lysing certain nonpathogenic organisms, its action on pathogens is very limited; however, it does destroy the tubercle bacillus (65).

Nasal secretions.—Factors affecting virus, but not clearly identified to date, are found in nasal secretions. Bell (66) has demonstrated a relationship between the antipoliomyelitis properties of human nasopharyngeal secretions and blood serum. This is in general agreement with the earlier findings of Francis (66a) in a study of the anti-influenza properties of human nasal secretions. A recent study of hemagglutinin inhibitor in nasal secretions by de St. Groth (67) bears on this problem. In unpublished work from our laboratory, nasal secretions from different subjects have been tested against pathogenic bacteria. These secretions vary markedly in their action on bacteria, depending on the subject from whom they were obtained. There is little doubt that in samples from the same subject variations occur which may be influenced by age, presence of inflammation, and immunological history of the host. Also the bacterial flora of various body cavities, such as the nose, mouth, and gut, must be considered in explaining the antimicrobial properties of the secretions of these parts. As early as 1915, Colebrook (68) noted the apparent antagonistic action of pneumococci and streptococci on the meningococcus. He found that the antimeningococcal factor produced by these organisms was filterable and that it was inactivated between 70°C. and 80°C. Since then, others have also demonstrated antagonisms between organisms frequently found in the host. Dold & Weigman's observation of the antagonistic effect of human saliva on the diphtheria organism (69) has been extended by Thompson & Johnson (70) with the finding that this antagonism is attributable to the presence of *Streptococcus mitis*. Thus, the importance of bacterial ecology as possibly affecting host resistance is suggested. The disturbing effect of antibiotics on host resistance as related to bacterial flora will be reviewed in connection with gastrointestinal defense mechanisms.

Gastrointestinal tract secretions.—The gastrointestinal tract is exposed not only to microorganisms ingested with food, but also to those which impinge on the mucus layer of the upper and lower respiratory tracts or are trapped in the mouth as man breathes, and ultimately are swallowed. It is fortunate that the gastrointestinal tract is supplied with a variety of defense mechanisms, including body fluids possessing antimicrobial properties.

Saliva has been studied in the past for its antimicrobial properties, and the results of the various investigations have been reviewed by Thompson (71). It is apparent that saliva contains, besides lysozyme, another group of

antimicrobial factors which may be related to the bacterial flora (72). This conclusion has been supported by other studies (73, 74). Here again, it is well to bear in mind that the mechanical flow of tears, nasal secretions, bronchial and tracheal secretions, and saliva may in itself be a very effective defense mechanism moving the organisms to the gastrointestinal tract for destruction or elimination.

Gastric juice owes part of its germicidal activity to its acidity, part to the enzyme pepsin, and part to factors which have not yet been adequately identified. Unpublished studies in our laboratory indicate that gastric juice of both animals and man contains a factor which is neither acid, pepsin, nor lysozyme, yet which acts on various bacteria.

The infrequency of severe infections of the pancreas has not stimulated immunologists to date to study adequately the natural defense mechanisms of this organ or its secretions. However, Paoletti (75) has studied the resistance of the pancreas to tuberculosis and believes that there is a bactericidal factor associated with the protein of pancreatic extracts. On the other hand, the biliary passages which lead into the intestinal tract, along with the pancreatic duct, are frequently infected, causing serious disease in the patient. The effect of bile acids on microorganisms has received considerably more attention than that of pancreatic secretions, but even here, bile as it comes from the liver has not been studied thoroughly. A review of pertinent literature, beginning with Neufeld's critical observations in 1900 on the antibacterial effect of bile on living pneumococci, has been extended by Stacey & Webb (76). These authors observed a relation between the effect of the bile acids and their surface tension-lowering effects, an observation which others may challenge.

The intestines contain food in various stages of digestion, digestive juices with recognized and unrecognized antimicrobial factors, and tremendous numbers of microorganisms. The organisms all are potential sources for antibiotics against other organisms. Truly a treasure house for the investigator of host resistance who likes his problems complicated! Recent consideration by the gastroenterologists of lysozyme as a possible cause of ulcerative colitis has given way to a belief that this enzyme represents a reaction to injury rather than an etiologic factor in this disease (77). The mucus secretions, aside from supplying a mechanical protection for the intestinal mucosa, appear to possess antimicrobial properties against certain pathogens. For example, Becker (78) has shown that autoclaved duodenal mucus from chicks 54-days-old inhibits the growth of Ascaridia. This effect increases with the age of the fowl, reaching a maximum at 125 days, and it may represent a mechanism explaining the increased resistance of this host with advancing age. Recently it has been shown (79) that a mucopolysaccharide obtained from the intestinal tissue of mice inhibited the infectivity of Theiler's GD VII strain of virus and the Lansing strain of poliomyelitis. Very little of this material was found in the intestines of man, monkey, rabbit, rat, hamster,

sheep, cow, or pig. Furthermore, an enzyme was recovered from the feces of mice which was capable of destroying the protective properties of this mucopolysaccharide.

The common use of antibiotics, particularly those administered orally, has resulted in complicating infections thought to arise from drug-resistant organisms in the intestinal tract (80 to 83). These infections, some of them fatal, have drawn attention to the role of the normal flora of the host in preventing the growth of organisms potentially dangerous when present in large numbers. Paine (84) has clearly demonstrated, *in vitro*, the inhibitory effect of *Escherichia coli* on monilia and the rapid growth of monilia organisms in cultures inoculated with both *E. coli* and the monilia when the growth of the former was inhibited with (chlortetracycline) aureomycin. These *in vitro* findings have been confirmed by tests in children (85). When the patients were given chloretetracycline or chloramphenicol (chloromycetin), the incidence of *Candida albicans* in the intestinal tract rose from 17 per cent before treatment to 33 per cent afterward, with a concomitant decrease in gram-negative and gram-positive organisms. Similar studies have been carried out with the drugs neomycin and oxytetracycline (86). This problem has become serious enough to call for its designation as a "New Clinical Syndrome."

Antibiotic factors produced by various pathogenic or nonpathogenic organisms found in disease or as part of the normal flora of man have been reported with increasing frequency. The earliest such observation dates back to work in Pasteur's laboratory. Recently an antibiotic has been demonstrated in medium in which *Micrococcus tetragenus*, often found in the upper respiratory tract, had been grown (87), which inhibited *Mycobacterium tuberculosis* and some gram-positive and gram-negative organisms. The antibiotic-producing properties of ocular organisms have been demonstrated (88). The active factor in most instances was a polypeptide. Bacteria have been shown to have an inhibitory effect on the growth of *Trichomonas vaginalis* (89).

It is apparent that in the investigation of host resistance it is quite important to consider the part played by antibiotic substances produced by the normal flora of the host. Even as large geographical areas are affected by the natural conflicts of plants and animals, ecological balance of microbial forces affects the host. The matter of cooperation and conflict among primitive organisms is well developed by Burkholder (90).

The genito-urinary tract secretions.—The genito-urinary tract produces fluids which, besides mechanically washing away microorganisms, possess some antimicrobial properties. The acid reaction of the vagina, attributable in part to its flora, definitely limits the number and kinds of pathogens which can survive in this organ. The secretions of glands in the genital tracts have been studied to a limited degree for germicidal activity. Recently, Taylor & Morgan (91) have demonstrated two antibacterial factors in human semen and prostatic fluid. One resisted heating at 100°C. for 30 min. and was

active against *Staphylococcus aureus*, *E. coli*, *Neisseria gonorrhoeae*, *N. meningitidis*, and *Bacillus cereus*. The other factor was heat-labile, being destroyed at 100°C. in 10 min. Its antimicrobial activities indicated that it was a lysozyme. Rozansky, Gurevitch & Brzezinsky (92) have shown that 80 per cent of specimens of human semen were bacteriostatic for several strains of *S. aureus*. The active principle was not destroyed at 56°C. in 30 min.

An interesting finding of a bacteriostatic effect of human urine on *M. tuberculosis* has been reported by Björnesjö (93). The recognized biochemical constituents of urine were not responsible for this effect. The factor was characterized as nonvolatile and resistant to acids and boiling. It was soluble in water and absolute alcohol but not in carbon tetrachloride, chloroform, or petroleum ether, and it was readily dialysed through cellophane. On electrodialysis, it was concentrated in the anode chamber.

Mammary gland secretions.—Milk has been known for many years to possess antimicrobial properties. In fact, fear of destroying such properties has been used by those supporting the sale of unpasteurized milk and by pediatricians favoring breast feeding of infants. Work on the antimicrobial properties of milk, including the important studies of Jones, has been reviewed and extended by Wilson & Rosenblum (94, 95, 96). These investigators have further contributed to our knowledge of the subject through their studies of the factor, lactenin, found in cow's milk, which inhibits and kills hemolytic streptococci, particularly those belonging to group A. This same factor is found in human and goat milk. They point out some similarities between lactenin and Tillet's acute phase serum factor which also inhibits the growth of streptococci. Lactenin was found to be reversibly inactivated under anaerobic conditions produced by excluding oxygen or by such reducing agents as cystine, glutathione, or thioglycollic acid. The authors believe that lactenin plays a role in preventing widespread epidemics from milk-borne streptococci. Lactenin withstands pasteurization, but not the temperature of 80°C. or higher used in canning milk. In the host, however, lactenin fails to prevent streptococcal infections because of the low oxidation potential in the mammary gland. It also fails to protect mice against intraperitoneal or subcutaneous inoculations of streptococci.

Skin and its secretions.—The skin is exposed to numerous pathogens and does real service in defending the host. The mechanical integrity of the skin is in itself a most effective defense mechanism. Rarely can evidence be found of the invasion of the body through the unbroken skin. On the other hand, a skin without a break is most unusual. Earlier studies have been concerned with the so-called autosterilizing, or self-disinfecting, power of skin. These have been reviewed by Burtenshaw (97), who also showed that ether and alcohol extracts of skin possessed marked action against streptococci, *Corynebacterium diphtheriae*, and enteric organisms. Saline extracts were much weaker and less consistent in their action. He concludes that the long-

chain fatty acids and soaps are the chief, if not the only, bactericidal factors in skin. Current work (98, 99, 100) dealing with the physiology and chemistry of skin secretions in health and disease should make future studies of the antimicrobial properties of skin more fruitful.

Fungistatic action of adult hair fat was attributed (101, 102) to the high content of free, unsaturated fatty acids which inhibit the growth of *Microsporum audouini* in concentrations as low as 0.0002 per cent. Young individuals susceptible to this infection were claimed to secrete less active fungistatic substances than do older people. In more recent work, Kligman & Ginsberg (103) failed to confirm this observation and gave the opinion that the difference in susceptibility of children and adults to this fungal infection was not as marked as generally believed. They speculated that differences in host susceptibility might better be searched for in the hair shaft rather than in the secretions.

Tissue Extracts

In another discipline, hematology, the blood has been referred to as the mirror of the tissues. A great deal can be learned of the functioning of the hemopoietic tissues by examining the peripheral blood. To a degree, this is true in studying host resistance. However, we must look further than blood or other body fluids to get a more complete picture of all that is involved in host resistance. The tissues of a mammal are complex morphologically, biochemically, and physiologically. It is with no small envy that the investigator concerned with mammalian host resistance looks over the shoulder of his colleague studying the resistance of a bacterium to bacteriophage. Nevertheless, a start has been made in investigating the role of tissues as such in host resistance.

There are various ways to study the role of the different tissues in the pathogenesis of an infectious disease. A test organism can be inoculated into animals intravenously, or by a more appropriate route if indicated. The animals can then be sacrificed at intervals and their tissues examined microscopically after routine or special staining. Electron microscopy with particularly thin tissue slices may be used if small particles such as viruses are to be studied (104). Tagged antibodies may be used in tracing the distribution of viruses (105). Cultures of the tissues may be made if survival of the bacterial pathogen is of concern to the investigator, and it usually is in studying host resistance. This general approach has given very useful information in understanding such phenomena as the pathogenesis of pneumococcus pneumonia (106, 107), the initial accumulation and later destruction of bacteria in the spleen, liver, and other organs (108), the spread and survival of viruses in host tissues (109, 110, 111), and the reaction of tissues of primates in defending the host against malarial parasites (112).

Certain tissues thought to be concerned with resistance, such as the spleen, may be removed and the result on the over-all resistance of the host then determined by challenge with graded doses of the pathogen in question.

Such a technique has led to an appreciation of the role of the spleen in host resistance to such pathogens as *Haemobartonella muris* (113). Splenectomy has been reported to profoundly depress the resistance of a vole, *Microtus guentheri*, to *Plasmodium berghei* (114). On the other hand, splenectomized rats have been found to be only slightly more susceptible to *Trypanosoma lewisi* infections than are normal animals (115). The effect of removal of the spleen was more marked if the animals were challenged within a day or two after the operation, which suggests a compensating increase in other body defenses. Lowering of resistance of human infants subjected to splenectomy for therapeutic reasons has been suggested by a resulting overwhelming bacteremia following such a procedure (116).

During the past ten years, interest in the germicidal properties of tissue extracts has been revived. New techniques for separating and identifying such extracts, at least partially, have been developed. The fact that the host-parasite relationship depends to a considerable degree on reactions at the chemical level between host cellular components and components of the parasite has been gradually accepted. Even as growth in the test tube depends on the presence of growth factors and absence of inhibitory substances, so it is with the growth of organisms in the body.

The antimicrobial factors separated from tissues seem to vary both as to their chemical nature and their biological activities. In reviewing current studies, the reader is frequently left with such questions as the following: (*a*) What is the chemical nature of the antimicrobial factor? (*b*) Was the test microorganism virulent for the host from which the tissue extract was obtained? (*c*) Was the organ from which the extract was obtained resistant or susceptible to the test organism?

Water-soluble antimicrobial factors.—Miller and associates (117) found that histones prepared from calf thymus inhibited oxygen uptake of *Escherichia coli*, group A streptococci, *Salmonella typhi*, and Type I pneumococcus. However, these extracts did not appear to be germicidal. A protamine, salmine sulfate, was germicidal for the same test organisms. Related work of Bloom and colleagues (118), with dog leukocytes, hog pancreas, and calf thymus, resulted in the separation of a basic polypeptide with a high lysine content which was bactericidal *in vitro* against *Bacillus anthracis* and protected animals to a limited degree against infections with this organism. Similarly (119), a polypeptide was obtained from hog thyroid which inhibited oxygen uptake by *Micrococcus pyogenes* (var. aureus), a beta hemolytic streptococcus, and *Bacillus megatherium*. Tryptic digests (120) of human and bovine red blood cells have been found to possess a bacteriostatic action against gram-positive and gram-negative bacteria. Extracts of erythryocytes of other species were less active. About 0.12 mg. per ml. was the minimal effective dose *in vitro*. The authors believed the active principle to be a peptide. It is unfortunate that these extracts were not tested against parasites which invade erythrocytes. Also the question remains as to whether or not the active principle resulted from the digestion procedures employed in

obtaining it. Nutini and associates, in studies which were later reviewed, confirmed, and extended (121), investigated the antibacterial effects of aqueous extracts of various tissues from different hosts. Extracts of brain and spleen were particularly effective against hemolytic streptococci and staphylococci, but not against pneumococci. The enteric organisms were stimulated rather than inhibited. These extracts, which were active in concentrations as low as 1:1,000, favored the accumulation of coagulase-negative white variants from coagulase-positive *Staphylococcus aureus*. When administered to mice infected by intracutaneous, intraperitoneal, or intravenous inoculation of the virulent staphylococcus, these extracts reduced the mortality from 90 to 2 per cent.

Recently (122) it has been shown that saline extracts of normal rat spleens, freed of cells and larger particles by centrifugation and filtration, would inhibit growth *in vitro* of strains of the tubercle bacillus which were avirulent (B.C.G.) or virulent (H37Rv) for guinea pigs. The rat, of course, has a rather high resistance to the tubercle bacillus. It would be interesting to have comparative data on the relative effectiveness of extracts of organs from several hosts varying in their susceptibility to *Mycobacterium tuberculosis*. A thermostabile factor bactericidal for tubercle bacilli has been found in aqueous extracts of bovine spleen, lung, liver, kidney, and muscle (123). It is not possible to classify this material except insofar as it was water-soluble, thermostabile, and was adsorbed on activated charcoal. Since it could be eluted with acetic acid, it might, of course, be a basic peptide.

The antibacterial action of spermine has received recent critical attention by Hirsch & Dubos (124). The authors reviewed the pertinent earlier references to this material, from the first observation of it as crystals in stored human semen, by Leeuwenhoek in 1678, to the determination of its structure, in 1926. This organic base $H_2N-(CH_2)_3-NH(CH_2)-NH_4-(CH_2)_3-NH_2$ has been currently isolated, by Hirsch & Dubos, from various animal tissues such as beef kidney, and some of its physical, chemical, and antimicrobial properties have been determined. They found it to be bacteriostatic for virulent and avirulent strains of *M. tuberculosis*, both human and bovine, and discussed its possible role in host resistance to this pathogen. Not only is this material not bacteriostatic for the nonacid-fast organisms studied, but it may actually stimulate their growth and protect them against the antagonistic action of quinine, quinocrine, and stilbamidine. In subsequent studies, Hirsch (125), found that the antimicrobial properties of spermine depend on a serum factor. This factor, associated with the α globulin, appears to have an enzymatic action on spermine, thus producing the active tuberculostatic substance.

Dubos & Hirsch (126) have recently isolated a basic peptide from different bovine tissues, particularly calf thymus, which over a period of several weeks significantly decreased the survival of strains of *M. tuberculosis*. There was little correlation between its effect on the organisms and the assumed virulence of these strains for the host. It had no *in vivo* effect on the development of tuberculosis in mice. This material, although similar to that pre-

viously isolated from calf thymus by Bloom and his colleagues (118), differed in arginine content, in behavior on dialysis, and in ultraviolet light absorption from that described by Hirsch & Dubos (127). Since this material was extracted at an acid pH, the authors suggested the possibility that it may not exist as such in the thymus but may arise during extraction from hydrolysis of a histone. The possibility that any antimicrobial factor extracted from tissues may be an artifact, even when mild procedures are employed, must always be borne in mind.

There is an increasing interest in peptide linkages of substances having specific physiological effects, such as gramicidin, insulin, glutathione, etc. From current reviews (128) it would appear that these substances may owe their peculiar physiological activities, in part at least, to the internal structures of their polypeptide chains. For example, gramicidin appears to have a cyclic chain and glutathione a branched chain, in contrast to the usual open polypeptide linkage of proteins. Thus the immunologist searching for chemical substances which contribute to the host's resistance will do well to select extraction methods likely to produce minimum change in the finer structure of the substances for which he searches.

Antimicrobial factors of lipid nature.—In a recent comparative study of extracts of tissues of guinea pigs, Henley (129) isolated a lipid material which inhibited the growth of *Diplococcus pneumoniae* Type I. It was found most abundantly in guinea pig liver extracts and to a much less degree in rat livers. The guinea pig was highly resistant and the rat susceptible to the test organism. Although the spleen had less of this material, again it was most readily demonstrated in spleens of the resistant guinea pig. Extracts of brain tissue of both animals had very little pneumococcidal properties. The active extracts which were not completely purified were weakly soluble in water, soluble in 50 per cent ethanol and acetone. The extracts resisted boiling but were inactivated by normal serum. The factor was proved not be to bile or cholesterol, but was thought to be related to free fatty acids or fatty-acid esters. During the fractionation procedures, quantitative evidence was obtained which suggested that the antimicrobial action of this material might be inhibited by other substances in the tissues, and that the resulting *in vivo* action would depend on a balance of these two substances or groups of substances. Clark and his colleagues (130) have demonstrated that lipids obtained from brain or placenta tissue would inhibit virus hemagglutination.

Besides searching for antimicrobial extractives from organs, investigators have recently turned to the ground substance, the hyaluronic acid polymers that bind tissues together. This is an interesting approach to the problem of resistance, since many pathogens elaborate the enzyme, hyaluronidase, which is capable of hydrolyzing this material. Thus, if antimicrobial substances arose during such hydrolysis, this result might act to the advantage of the host. This point is made by Duran-Reynals and his colleagues (131, 132) in discussing results of studies on the action of hydrolyzed and nonhydrolyzed hyaluronic acid preparations against vaccinia virus.

Tissue metabolites affecting growth of pathogens.—Until recently, the main

emphasis in searching for tissue properties which might influence the infectious process has been placed on germicidal effects of tissue extracts. However, attention is now being directed by some investigators to altering the resistance of the host to certain strains of bacteria by injecting a growth factor for the pathogen. In studies with *para*-aminobenzoic acid (PAB), Bacon, Burrows & Yates (133) found that this material was not present in sufficient concentration in the tissues of the mouse to support growth of the test organism, a variant of *Salmonella typhosa*, which lacked the ability to synthesize PAB.[2] When this material was injected, or when a relatively avirulent bacterium capable of synthesizing PAB was inoculated with the test organism, the mice died.

It has also been shown (134) that D-alanine *in vitro* changed the population of a culture of brucella to an avirulent type. *In vivo* tests of the effect of administration of DL-alanine to brucella-injected guinea pigs showed that, instead of increasing resistance of the host, the material acted in the opposite manner (135). The authors suggested that this effect was associated with the development of virulent S′ alanine-resistant variants. Specific antiserums against S′ inhibited this increase in virulence, and this result tends to support the authors' hypothesis. The inhibitory effect of L-methionine and DL-ethionine on the propagation of influenza virus in tissue cultures has been clearly demonstrated by Ackermann (136). Ethionine has been shown to have some protective action when fed to mice infected with Semliki Forest virus (137). In these experiments, 52 per cent of treated animals survived as compared to 20 per cent of the controls. The field of antimetabolites in general is an active one, and the immunologist interested in the effects of antimetabolites on host resistance to infectious diseases or to tumors will be interested in Shaw's current review of the subject (138).

Phagocytosis

The phagocytic cells play a most important role in the defense of the body against bacteria, protozoa, and probably viruses. Several fairly recent reviews (139, 140, 141) have been published. Although both the circulating and fixed phagocytic cells are concerned with this defense mechanism, it is the latter, found particularly in the spleen, lymph nodes, liver, lungs, and connective tissue throughout the body, that are especially significant in host resistance. The effectiveness with which these cells operate can be demonstrated by inoculating *E. coli* intravenously into a test animal. The bacterial count of the blood may drop from a million bacteria per ml. to practically zero within 30 min. Animals sacrificed at that time may show large numbers of bacteria in the spleen, liver, and lungs. This type of experiment, suggested by Bull (108), demonstrates the activity of the reticuloendothelial system, a functional if not a true anatomical system. The clearance rate of bacteria from the blood as well as from the tissues depends on the parasite's virulence for the host under consideration, as well as the host's natural resistance or induced immunity. Highly virulent organisms injected intravenously into a

host will at first decrease in numbers, only to increase thereafter and ultimately kill the animal. The effect of immune serum in stimulating blood clearance is great and has recently been studied quantitatively (142). Not only bacteria, but also other pathogens such as viruses (143) and protozoa (144), are cleared from the blood stream by the reticuloendothelial system. A current study (145) of the phagocytosis of influenza virus, in which the action of polymorphonuclear leukocytes was blocked by treatment with periodate, suggests a receptor site on these cells similar to that on erythrocytes.

Considerable attention is now being directed to studies of the activities of the fixed phagocytic cells. Their role in defending the host against streptococci has been studied (146). The activity of these cells in the presence of bacteremia, even when the animal was in a terminal stage of a pneumococcus infection, has been demonstrated (147). Also in rabbits with profound damage to the blood-forming organs as a result of benzene poisoning, the splanchnic removal of *Micrococcus aureus* from the blood stream was normal (148). That other host cells not usually considered phagocytic may engulf particles is emphasized by Altschul & Friesen (149), who demonstrated that skeletal muscle would take up foreign particles under some conditions.

Not only are the phagocytic cells intimately involved in freeing the host's blood and tissues of microorganisms in health and disease, but they are also concerned with antibody production, the restriction of infectious processes, the clearing of inflamed tissue as seen in the resolution of pneumonia, and possibly the promoting of tissue growth in host repair processes. What phagocytes do is obvious, how they do it is not always clear. There are many points of similarity of action between phagocytes and amebae. In fact, the circulating phagocytes have been referred to as the "amebae of the blood."

The maintenance of an adequate concentration of circulating phagocytes in the blood is important for the defense of the host. In the presence of infection, these cells increase in number in the circulating blood and infiltrate the infected area. Menkin has made significant studies of chemical constituents in inflammatory exudates which might favor the accumulation of phagocytic cells. He has reviewed his theories and facts supporting them in a recent book (150). From such exudates he has isolated several substances which influence phagocytic cell accumulations in areas of inflammation. One substance, a leukocyte-promoting factor, appears to be liberated by injured cells. This factor, designated L.P.F., is thermolabile and nondiffusible, and causes a sharp increase in circulating phagocytes by stimulating the growth of granulocytes and megakaryocytes in the bone marrow. It is associated with the α^1 and α^2 globulins of the exudate and may be a polypeptide combined with the protein. It has also been shown that peptides with a chain length of 8 to 14 amino acids obtained from enzymatic digested fibrin were able to induce swelling of the endothelium of blood capillaries. Obviously, the frst step toward contact between parasite and phagocyte must be the presence of phagocytes. Thus the L.P.F.,[2] by stimulating the production of poly-

morphonuclear leukocytes, favors this first step. The origin of other phagocytic cells which eventually accumulate at the site of inflammation is less well understood. They may migrate to the scene of action, or they may be formed from existing cells under stimulation of some chemical product of the inflammatory reaction.

Menkin believes that capillary permeability is increased by the action of a substance, which he has crystallized, designated as leukotaxin. This material is not histamine, hyaluronidase, or acetylcholine, and its function seems to be to get the leukocytes which have been acccumulated in the blood by L.P.F. into the tissue at the site of trauma. Leukocytes in man have a life span of from 12 to 14 days, as shown by tagging experiments (151), and they circulate in the blood for 9 to 10 days. The possibility of a nervous-humoral control has been suggested (152). Mechanical or electrical stimulation of the hypothalamic region resulted in a marked neutrophil leukocytosis, with a shift to the left indicating increased bone marrow activity.

The importance of a suitable surface on which the free phagocytes can move has been reported by several workers in the past. More recently Wood and his colleagues (153) have re-emphasized the importance of this physical condition for phagocytosis. In fact they report that highly virulent encapsulated pneumococci can be phagocytized in the absence of immune opsonins if there is a fibrin net to aid the phagocyte in trapping the bacterium. Fortunately for man, the inflammatory exudate in pneumococcus infections contains a great deal of fibrin. Less effective as a resistance mechanism would be the fibrin strands which might be laid down in a hemolytic streptococcus infection because of their early lysis attributable to the streptokinase production of these organisms.

Chemotaxis.—Once circulating phagocytes have accumulated in the bloodstream in adequate numbers and have migrated from the blood capillaries into the infected area, there are three essential steps in their destruction of bacteria: contact, engulfment, and digestion.

Establishment of contact between parasite and phagocytic cells may be based on chance or may involve a positive attraction of the phagocytic cells to the parasite, a phenomenon known as chemotaxis and well reviewed by McCutcheon (154). Not only polymorphonuclear leukocytes but also monocytes (155) are subject to chemotaxis and react in much the same manner. It has been generalized that the majority of bacteria exhibit leukotaxis *in vitro*. Filtrates of the gram-negative organisms produce substances that adversely affect this phenomenon (156). This has been confirmed by studies of carbohydrate complexes from gram-positive and gram-negative organisms (157). However, as in all host resistance problems, it is wise to define the problem specifically in terms of the species of host and the species and strain of parasite. The effects of pathogen virulence on leukocytic migration have been compared (158) using tubercle bacilli virulent or avirulent for the host from which the leukocytes were obtained. The virulent organisms inhibited chemotaxis, and avirulent ones did not. In a similar manner (159), virulent

brucellae inhibited migration of leukocytes obtained from normal animals, and avirulent strains did not. However, leukocytes obtained from infected animals, even when infection had existed for only 1 hr., were able to migrate toward the virulent organisms. The authors interpret this reaction as related to the general stress phenomenon.

Certain factors have been found to interfere with chemotaxis and thus with host resistance. Alcohol in concentrations approximating blood levels of the comatose state of alcoholic intoxication practically eliminates any movement of phagocytes (160). Cromartie *et al.* (161) have demonstrated a "tissue damaging factor" present in the edema fluid of anthrax lesions. This material was later characterized as a substance with high electrophoretic mobility and possibly related to, but not identical with, the glutamyl polypeptide of organisms grown *in vitro*. It damaged the tissue collagen and either destroyed leukocytes or prevented their accumulation in the lesion. Thus, the work on bacterial aggressins begun by Bail has been materially advanced. Knisely (162) suggests that the contact between blood-borne pathogens and the fixed phagocytic cells, at least those in the spleen and liver, is increased not only by its passing through a large capillary bed, with resulting slowing of its flow, but by actual momentary stoppage of flow by a valve mechanism in the organ capillaries.

Engulfment of pathogens.—The second stage of phagocytosis begins after the establishment of actual contact between the parasite and the cell. Virulent bacteria frequently resist repeated efforts of the phagocytic cell to ingulf them. Parasite virulence is sometimes correlated with its capacity for capsule formation; often it is implied that the capsule mechanically prevents phagocytosis. But the reaction must be more complicated than this, and it may well be of a biochemical nature. Preisz many years ago demonstrated that *Bacillus anthracis* variants formed differing capsules, one of which contributed to virulence, while a second type did not. Furthermore, the encapsulated forms of pneumococci Type I are not virulent for guinea pigs although they are for the mouse and rat. Finally, Felton & Bailey (163) have shown that animal resistance to pneumococci may be lowered by injecting a solution of capsular substance. This strongly suggests that the capsule's action does not depend on capsular structure per se but rather on its chemical composition, which interferes with phagocytic function. There are other substances which inhibit phagocytosis or actually destroy the phagocytes. Some bacterial filtrates have been said to possess leukocidins or aggressins, and these of course, whatever they may be chemically, in one way or another decrease phagocytosis. The mumps and influenza A viruses will reduce the action of guinea pig phagocytes on bacteria (164). Since the guinea pig is not susceptible to either virus, it would be interesting to know what effect these viruses would have on the phagocytic cells from a susceptible host. It has been demonstrated that the purified C-reactive protein found in the serum of patients with a wide variety of infectious and noninfectious diseases will, in a final concentration greater than 0.005 mg. per ml., destroy normal

human leukocytes (165). An unfortunate situation indeed, in which the host elaborates a material during an infection which interferes with phagocytosis! Mucus secretions also contain afactor which in some manner appears to interfere with the defense activities of the phagocytic cells. Recent studies indicate that this factor may be a heparin (166). Again, not all that the host produces may be to its advantage in defending itself against infection.

Since the classical studies of Hamburger, published in 1904, further studies have been made on factors favorably affecting phagocytosis, other than normal or immune opsonins. It has been claimed that hyaluronidase will increase phagocytosis as much as 60 per cent (167). The heat-inactivated enzyme did not have this effect. The role of surfaces in increasing phagocytosis of virulent encapsulated bacteria in the absence of immune serum has been enlarged upon by Smith, *et al.* (168). The observation (169) that formalin-killed *E. coli* were phagocytized more readily after previous contact with filter paper or *para*-hydroxybenzoic acid has raised some doubt as to the role of surfaces per se in surface pharocytosis.

Since it is generally recognized that inadequate nutrition tends to lower host resistance to bacterial infections, it is of interest to note the results of inadequate food intake on phagocytosis. It has been reported that in birds with protein deficiency, pneumococci were cleared from the blood by the reticuloendothelial system less readily than in the normal host (170). On the other hand, in rats after a 36-hr. fast, phagocytosis was increased over that in the fed animals (171). There is more agreement now on the effect of ascorbic acid deficiency in decreasing phagocytosis (172, 173). Of incidental interest, a possible relationship between the eosinophilic reactions to stress and the ascorbic acid level in rats has been suggested (174). Contradictory results have been published on the effect of cortisone on the activity of the fixed phagocytic cells in taking up inorganic colloids. Gell & Hinde report no effect (175), while Marcus, Hill, and Esplin (176) find a stimulating effect.

Substances unrelated to natural infections have also been shown to increase phagocytosis. Such substances have been referred to as "artificial opsonins" by earlier investigators. It has recently (177) been claimed that *E. coli* grown in beef infusion was more readily phagocytized than organisms from tryptose medium. It was believed that the opsonic factor was not present as such in the medium, but was developed by the organism in response to a stimulating factor in the medium. There might well be other interpretations for these findings, such as the effect of the medium on the virulence of the bacterial population.

It is interesting that the only therapeutic agents capable of affecting the surfaces of bacteria so that phagocytosis is significantly increased are the antibacterial serums. No available chemotherapeutic drug or antibiotic is able to increase this resistance mechanism. That substances with such activity may be found is indicated by a recent report that certain relatively simple nonbacteriostatic quaternary ammonium compounds increase phagocytosis in the test tube and in the animal, and protect the animal against

an otherwise lethal inoculation of pneumococci (178). The value of using drugs having opsonic properties with chemicals which have bacteriostatic characteristics is obvious. It is not sufficient simply to prevent parasite growth, if the patient is to be cured. The mechanisms of host resistance must destroy the organism, and this is primarily the responsibility of the phagocyte.

Some attention has been given to the effect of the autonomic nervous system and the related endocrine organs on phagocytosis. The more pertinent literature on this subject has recently been reviewed (179). The authors of this review have also reported their own studies of the effect of splanchnic stimulation in increasing the opsonic properties of serum. They concluded that stimulation of the sympathetic system increased phagocytosis partly by producing changes in the blood serum and also by direct action on the phagocytes. A comprehensive review of the literature bearing on the relation of the hypophysis-adrenocortical system to phagocytosis has been recently published (180).

We know some of the factors which affect phagocytosis favorably or unfavorably; however, there is no clear explanation of the actual mechanisms involved in phagocytosis. Probably they involve physical phenomena of a local nature, occurring in part of the surface of the phagocytic cell rather than its entire outer membrane. These subtle changes have been missed in electrophoretic measurements which represent a summation of electrical surface charges of the entire cell. This is illustrated by results from our laboratory (181) showing that both bacteria and leukocytes are negatively charged. The quantitative value for the electromobility of streptococci suspended in normal guinea pig serum was 0.6 μ/sec/volt/cm., while that of guinea pig leukocytes was 1.0 μ/sec/volt/cm. Hence, a theoretically impossible situation exists in which particles of similar magnitude and type of charge (negative) are attracted to one another. Moreover, available data are not adequate to support Fenn's theory (182) that phagocytosis results because of interfacial tension changes. However, an opinion supporting Fenn's early theory has recently been published (183).

Enzymology of phagocytes.—The final and crucial step in phagocytic defense involves the digestion of the parasite, or at least its destruction. This step is critical in an effective defense process. An understanding of it is predicated on general phagocyte enzymology and specific information regarding the host-parasite relation. To date very little of the work done on phagocyte metabolism has been directed to the problem of host resistance. Therefore, one must reason for fragmentary data attempting to apply the results of metabolic studies to an understanding of this phase of host resistance. Biochemically, pathogens are very complex, and they usually contain carbohydrates, lipids, and proteins which may be vulnerable to the enzymatic processes of the phagocyte. In turn, these same substances in the parasite may stimulate or inhibit enzyme processes essential to the functioning of the phagocyte if it is to destroy the pathogen.

A beginning in the study of the enzymology of phagocytes was made in 1910 by Opie. The classical studies of Levene and Meyer about this same time established the anaerobic potentialities of these cells. Since then, other investigators have contributed to our knowledge of the various enzymes present in leukocytes. More recent contributions to our knowledge of these enzymes have been made by Rebuck (184) and Barnes (185), and leukocyte metabolism has been investigated by Valentine (186) and by McKinney and colleagues (187).

Of some importance is the observation that leukocytes have about 2000 times as much β glucuronidase in or on them as is contained in serum (188, 189). The probable role of this enzyme in detoxifying toxic products arising in the infectious process deserves further attention.

The lipolytic enzymes of phagocytes are of special interest since lipids are present in many pathogens, especially the acid-fast organisms. Current opinions, published (190) and unpublished, expressed by students of host resistance to tuberculosis, suggest that immunity to this disease may be related to changes in the enzyme systems of the resistant host rather than to circulating or tissue antibodies. Unfortunately, current studies in this field are far from numerous. The studies of Wong on esterases of leukocytes (191) at least keeps alive an interest in this subject.

The effect of pathogens, fractions of microorganisms, or products of the infectious process, arising from the tissues or infiltrating cells or fluids, on host tissue metabolism is now receiving attention. The effect of herpes virus infection on the metabolism of embryonic liver and heart has been studied (192). In this investigation, it was noted that virus proliferation in host cells stimulated nucleic acid synthesis and changed the normal ratio of succinoxidase to ketoglutamic acid activity. Glycolysis by homogenates of normal mouse and rat brain has been compared with that of brain infected by meningo-pneumonitis virus (193). These two hosts vary in their susceptibility to this virus, the rat being much more resistant to intracerebral inoculation. Glycolysis in the homogenate of infected rat brain was completely inhibited in 90 min., which was not true of normal rat brain or normal or infected mouse brain. Earlier studies demonstrated differences in the metabolism of normal and infected tissue. More recently (194) it has been shown that guinea pigs infected with H37Rv or BCG strains of *Mycobacterium tuberculosis* showed a decrease in succinic dehydrogenase activity of kidney tissue, and to some extent the same held for spleen and liver tissue. A general decrease in glycolytic function of mouse lung after the development of consolidation as a result of mouse pneumonitis virus or lipid pneumonia has been demonstrated (195). Still another finding bearing on changes in the metabolism of traumatized tissue is that concerned with the injection of guinea pig brain with an emulsion containing non-pathogenic acid-fast organisms. The histiocytes showed an increase in a lipolytic enzyme capable of producing demyelinization (196).

In developing a better understanding of the biochemical basis for host

resistance, however, it would seem that comparative metabolic studies on host reaction to virulent and avirulent strains of a parasite, or the reaction of a susceptible and resistant host to a virulent organism, would be most desirable. Alonso (197) has shown that the anaerobic metabolism of rat leukocytes was markedly stimulated by pneumococcus type I capsular substance (SI). However, glycolysis of guinea pig leukocytes was stimulated only slightly, in the 1.5-hr.-period of observation. Under aerobic conditions the capsular substance stimulated oxygen uptake of guinea pig leukocytes about ten times as much as it did in the case of leukocytes from the rat, which was highly susceptible to the test organism *Diplococcus pneumoniae* Type I.

In another approach to the problem of the biochemical basis for host resistance, Murayama (198) has studied the effect of pneumococcus SI on the activity of the adenosinetriphosphatase from leukocytes of the resistant guinea pig and the susceptible rat. He found that SI increased the activity of adenosinetriphosphatase from guinea pigs and decreased rat leukocyte adenosinetriphosphatase action. In a personal communication, he has stated that SIII increases adenosinetriphosphatase activity of rabbit leukocytes and SI decreases the activity of the same enzyme preparation. The rabbit is resistant to Type III pneumococcus and susceptible to Type I strains.

An interesting approach to the application of current knowledge of metabolism to the problems of host resistance has been made by Berry & Mitchell (199, 200). These investigators arrived at the hypothesis that substances such as sodium fluoroacetate, sodium malonate, or sodium arsenite, which interfere in one way or another with the tricarboxylic acid cycle, might also lower the resistance of mice to *Salmonella typhimurium*. The literature cited indicated that the injection of these compounds resulted in decreased multiplication of poliomyelitis or influenza virus in mice. The authors noted opposite effects with bacterial pathogens, a not unusual situation in comparative pathogenesis studies on viruses and bacteria. Infected mice injected at eight-hour-intervals with either the malonate, fluoroacetate, or arsenite died earlier than the controls.

Teleologically, phagocyte metabolism has as its purpose the destruction of pathogens. Although progress to date has not been sufficient to define the fundamental steps in this process, research has gone on from a pragmatic point of view. Rogers & Tompsett (201) have shown that while both virulent and avirulent staphylococci were phagocytized by human leukocytes, only the avirulent forms were readily destroyed by the phagocyte. Attention has been called (202) to possible errors in studying the viability of ingested bacteria arising from intracellular clumping of the bacteria resulting in decrease in plate counts without destruction of the organisms. In fact, evidence of ultimate destruction of the phagocyte by the engulfed virulent staphylococci was reported. It has been recognized for many years that phagocytosis of virulent tubercle bacilli by polymorphonuclear leukocytes might result in spread of the disease. Suter has recently perfected a technique for measuring

the multiplication of tubercle bacilli in macrophages. This procedure is well adapted to studying a variety of problems such as the penetration of antibiotics into cells (203), comparative resistance of species or individuals within a species, and the effects of immunization. A. A. Grover, in our laboratory, is at present completing a description of the comparative natural resistance of rats and guinea pigs to tuberculosis and also the effects of active immunization using this technique.

Phagocytosis may for one reason or another be reversed, that is, the particle may be egested. Wilson (204) has observed that the vacuole in which phagocytized streptococci are enclosed may be discharged to the outside. The organisms may or may not grow depending upon how long they had been in the vacuole in the phagocyte. This suggests a fairly slow destruction of the phagocytized organisms within the leukocyte. Doubtless this rate of destruction would depend on both the organism and the phagocyte studied.

Host resistance depends not only on the ability of the host to defend itself against the invasion and *in vivo* multiplication of the parasite, but also on the susceptibility of the host to the disease-producing mechanisms of the parasite. Unfortunately, we know far too little about the causes of the aches, pains, fever, loss of appetite, and even death that may result from an infection. Occasionally a vital tissue is destroyed, for example, the wall of the aorta in cardiovascular syphilis or the motor neurons in poliomyelitis. Although we don't know why this should be the case, at least some information can be gained from such observations. On the other hand, the aches, pains, and elevated temperature of influenza go unexplained. The cause of death in lobar pneumonia cannot be charged to loss of functioning lung tissue, since a surgeon can remove an entire lung without producing death. The septicemia *per se* is not the answer; the blood of rats with *Trypanosoma lewisi* infection, for instance, teems with parasites. Hence, the term *toxemia* is suggested as the cause of signs, symptoms, and death in pneumonia. Obviously this must ultimately be resolved into biochemical terms with respect both to the toxin and to its mode of operation.

LITERATURE CITED

1. Howie, J. W., *Brit. J. Nutrition*, **2**, 331–37 (1949)
2. Clark, P. F., *Ann. Rev. Microbiol.*, **4**, 343–58 (1950)
3. Schneider, H. A., *Am. J. Trop. Med.*, **31**, 174–82 (1951)
4. Pond, W. L., Davies, W. L., Smith, S. C., Elvehjem, C. A., Rasmussen, A. F., Jr., and Clark, P. J., *J. Bact.*, **64**, 583–92 (1952)
5. Henle, W., *J. Immunol.*, **64**, 203–36 (1950)
6. Jordan, R. T., and Duffy, C. E., *J. Infectious Diseases*, **91**, 165–72 (1952)
7. Price, W. H., *Proc. Soc. Exptl. Biol. Med.*, **82**, 180–84 (1953)
8. Taylor, C. E., *J. Immunol.*, **71**, 125–33 (1953)
9. Sulkin, S. E., Wallis, C., and Murphy T. P. Jr., *Proc. Soc. Exptl. Biol. Med.*, **84**, 184–89 (1953)
10. Cox, H. W., *J. Elisha Mitchell Sci. Soc.*, **68**, 222–35 (1952)
11. Harford, C. G., and Parker, E., *Federation Proc.*, **12**, 446 (1953)
12. Nungester, W. J., Klepser, R. G., and Kempf, A. H., *J. Infectious Diseases*, **71**, 57–60 (1942)
13. Kass, E. H., and Finland, M., *Ann. Rev Microbiol.*, **7**, 361–88 (1953)
14. Fischel, E. E., Kabat, E. A., Stoerk, H C., Skolnick, M., and Bezer, A. E., *Federation Proc.*, **12**, 442 (1953)
15. Shee, J. C., and Fehrsen, P., *Brit. Med. J.*, **II**, 82 (1953)
16. Ilavsky, J., and Foley, E. J., *Proc. Soc. Exptl. Biol. Med.*, **84**, 211–14 (1953)
17. Davidson, A. G., Fox, L., and Gold, J. J., *Ann. Internal Med.*, **38**, 852–62 (1953)
18. Hanan, R., and Overman, J. R., *Proc. Soc. Exptl. Biol. Med.*, **84**, 420–23 (1953)
19. Clawson, B. J., and Nerenberg, S. T., *J. Lab. Clin. Med.*, **42**, 746–48 (1953)
20. Sonkin, L. S., *Am. J. Hyg.*, **53**, 337–54 (1951)
21. Brown, J. H., Cook, K. M., Ney, F. G., and Hatch, T., *Am. J. Public Health*, **40**, 450–58 (1950)
22. Reyniers, J. A., Erwin, R. F., and Gordon, H. A., *Lobund Repts., No. 2* (University Press, University of Notre Dame, South Bend, Indiana, 162 pp., 1949)
23. Mackie, T. J., and Finkelstein, M. H., *J. Hyg.*, **32**, 1–24 (1932)
24. Casals, J., and Olitsky, P. K., *Science*, **106**, 267–68 (1947)
25. Utz, J. P., *Proc. Soc. Exptl. Biol. Med.*, **69**, 186–89 (1948)
26. Utz J. P., *J. Immunol.*, **63**, 273–79 (1949)
27. Pillemer, L., Ecker, E. E., Oncley, J. L., and Cohn, E. J., *J. Exptl. Med.*, **74**, 297–308 (1941)
28. Seifter, S., Dozois, T. F., and Ecker, E. E., *J. Immunol.*, **49**, 45–49 (1944)
29. Dozois, T. F., Seifter, S., and Ecker, E. E., *J. Immunol.*, **47**, 215–229 (1943)
30. Heidelberger, M., *Am. Scientist*, **34**, 597–610 (1946)
31. Rice, C. E., *J. Immunol.*, **70**, 497–502 (1953)
32. Bordet, J., *Traité de l'immunité dans les maladies infectieuses* 95 (Masson et cie, Paris, France, 720 pp., 1920)
33. Tilden, E. B., *Proc. Soc. Exptl. Biol. Med.*, **32**, 265–67 (1934)
34. Ginsberg, H. S., and Horsfall, F. L., Jr., *J. Exptl. Med.*, **90**, 475–95 (1949)
35. Bang, F. B., Foard, M., and Karzon, D. T., *Bull. Johns Hopkins Hosp.*, **87**, 130–43 (1950)
36. Jacox, R. F., *J. Exptl. Med.*, **92**, 101–11 (1950)
37. Adler, F. L., *J. Immunol.*, **70**, 69–78 (1953)
38. Rothbard, S., *J. Exptl. Med.*, **82**, 107–18 (1945)
39. McCarthy, K., and Germer, W. D., *Brit. J. Exptl. Pathol.*, **33**, 529–36 (1952)

40. Howitt, B. F., *J. Immunol.*, **64**, 73–85 (1950)
41. Handler, B. J., *Am. J. Hyg.*, **21**, 18–26 (1935)
42. Culbertson, J. T., and Strong, P. S., *Am. J. Hyg.*, **21**, 1–17 (1935)
43. Kidd, J. G., *J. Exptl. Med.*, **98**, 583–606 (1953)
44. Ward, H. K., and Enders, J. F., *J. Exptl. Med.*, **57**, 527–47 (1933)
45. Gordon, J., and Hoyle, L., *J. Pathol. Bact.*, **43**, 537–44 (1936)
46. Ecker, E. E., Pillemer, L., and Kuehn, A. O., *J. Immunol.*, **43**, 245–58 (1942)
47. Maurer, P. H., and Talmage, D. W., *J. Immunol.*, **70**, 135–40 (1953)
48. Blomfield, A. M., *J. Hyg.*, **50**, 126–35 (1952)
49. Dozois, T. F., Wagner, J. C., Chemerda, C. M., and Andrew, V. M., *J. Immunol.*, **62**, 319–31 (1949)
50. Ecker, E. E., Seifter, S., and Dozois, T. F., *J. Lab. Clin. Med.*, **30**, 39–50 (1945)
51. Pillemer, L., Ratnoff, O. D., Blum, L., and Lepow, I. H., *J. Exptl. Med.*, **97**, 573–89 (1953)
52. Marcus, S., and Donaldson, D. M., *Proc. Soc. Exp. Biol. Med.*, **83**, 184–87 (1953)
53. Bornemisza, G., Goreczky, L., Ludány, G., and Vajda, J., *Z. Immunitätsforsch.*, **109**, 225–28 (1952)
54. Goreczky, L., Ludány, G., Tóth, J., and Vajda, J., *Z. Immunitätsforsch.*, **109**, 118–23 (1952)
55. Ledingham, J. C. G., *Lancet*, **II**, 898–901 (1922)
56. Pettersson, A., *Zentr. Bakt. Parasitenk.*, **42**, 56–63 (1906)
57. Hiss, P. H., and Zinsser, H., *J. Med. Research*, **19**, 399–410 (1908)
58. Gratia, A., and Goreczky, L., *Z. Immunitätsforsch.* **93**, 18–26 (1938)
59. Petterson. A., *Z. Immunitätsforsch.*, **88**, 210–26 (1936)
60. Hench, M. E., *An Investigation of the Effect of Blood and Serum from Resistant and Susceptible Host Species on the Growth of Type I Pneumococcus* (Doctoral thesis, Univ. of Michigan, Ann Arbor, Mich., 1952)
61. Krueger, R. C., and Heidelberger, M., *J. Exptl. Med.*, **92**, 383–91 (1950)
62. Orlans, E. S., *Brit. J. Exptl. Pathol.*, **33**, 451–61 (1952)
63. Burdon, K. L., and McRoberts, C. C., *Federation Proc.*, **12**, 438 (1953)
64. Rabe, E. F., and Curnen, E. C., *J. Pediat.*, **38**, 147–53 (1951)
65. Myrvik, Q. N., Weiser, R. S., and Kelly, M. C., *Am. Rev. Tuberc.*, **68**, 564–74 (1953)
66. Bell, E. J., *Am. J. Hyg.*, **47**, 351–69 (1948)
66a. Francis, *Proc. Soc. Exp. Biol. Med.*, **48**, 116–17 (1941)
67. Fazekas de St. Groth, S., *J. Hyg.*, **50**, 471–90 (1952)
68. Colebrook, L., *Lancet*, **II**, 1136–38 (1915)
69. Dold, H., and Weigmann F., *Z. Hyg. Infektionskrankh.*, **116**, 158–70 (1934)
70. Thompson, R., and Johnson, A., *J. Infectious Diseases*, **88**, 81–85 (1951)
71. Thompson, R., *Arch. Pathol.*, **30**, 1096–1134 (1940)
72. Thompson, R., and Shibuya, M., *J. Bact.*, **51**, 671–84 (1946)
73. Thompson, R., *J. Bact.*, **41**, 77 (1941)
74. Van Kesteren, M., Bibby, B. G., and Berry, G. P., *J. Bact.*, **43**, 573–83 (1942)
75. Paoletti, A., *Acta Med. Ital. Malattie Infettive e Parassitarie*, **5**, 298–301 (1950)
76. Stacey, M., and Webb, M., *Proc. Roy. Soc.* (*London*), **134**, 523–37 (1947)
77. Janowitz, H. D., Hollander, F., and Garlock, J. H., *Bull. N. Y. Acad. Med.*, **27**, 402–3 (1951)
78. Becker, R., *J. Parasitol.*, **39**, 467–80 (1953)

79. Mandel, B., and Racker, E., *J. Exptl. Med.*, **98**, 399–415 (1953)
80. Bernhart, G., *Schweiz. med. Wochschr.*, **82**, 1335–37 (1952)
81. Brown, C., Jr., Propp, S., Guest, C. M., Beebe, R. T., and Early, L., *J. Am. Med. Assoc.*, **152**, 206–7 (1953)
82. Spink, W. W., *J. Am. Med. Assoc.*, **152**, 585–90 (1953)
83. Fairlie, C. W., and Kendall, R. E., *J. Am. Med. Assoc.*, **153**, 90–94 (1953)
84. Paine, T. F., Jr., *Antibiotics & Chemotherapy*, **2**, 653–58 (1952)
85. McGovern, J. J., Parrott, R. H., Emmons, C. W., Ross, S., Burke, F. G., and Rice, E. C., *New Engl. J. Med.*, **248**, 397–403 (1953)
86. Dearing, W. H., and Needham, G. M., *Proc. Staff Meetings Mayo Clinic*, **28**, 507–12 (1953)
87. Spicer, S., *Antibiotics & Chemotherapy*, **3**, 254–55 (1953)
88. Halbert, S. P., Swick, L., and Sonn, C., *J. Immunol.*, **70**, 400–10 (1953)
89. Lawlis, J. F., Jr., *Butler Univ. Botan. Studies*, **10**, 153–70 (1952)
90. Burkholder, P. R., *Am. Scientist*, **40**, 601–31 (1952)
91. Taylor, P. W., and Morgan, H. R., *Surg. Gynecol. Obstet.*, **94**, 662–68 (1952)
92. Rozansky, R., Gurevitch, J., Brzezinsky, A., and Eckerling, B., *J. Lab. Clin. Med.*, **34**, 1526–29 (1949)
93. Björnesjö, K. B., *Notiz. ist. vaccinogeno antituberc.*, **1**, 30–31 (1951)
94. Wilson, A. T., and Rosenblum, H., *J. Exptl. Med.*, **95**, 25–38 (1952)
95. Wilson, A. T., and Rosenblum, H., *J. Exptl. Med.*, **95**, 39–50 (1952)
96. Wilson, A. T., and Rosenblum, H., *J. Exptl. Med.*, **95**, 51–59 (1952)
97. Burtenshaw, J. M. L., *J. Hyg.*, **42**, 184–210 (1942)
98. Kile, R. L., Snyder, F. H., and Haefele, J. W., *Arch. Dermatol. u. Syphilis*, **61**, 792–99 (1950)
99. Suskind, R. R., *J. Invest. Dermatol.*, **17**, 37–54 (1951)
100. Dole, V. P., and Thaysen, J. H., *J. Exptl. Med.*, **98**, 129–44 (1953)
101. Rothman, S., Smiljanic, A. M., and Shapiro, A. L., *Proc. Soc. Exptl. Biol. Med.*, **60**, 394–95 (1945)
102. Rothman, S., Smiljanic, A. M., Shapiro, A. L., and Weitkamp, A. W., *J. Invest. Dermatol.*, **8**, 81–98 (1947)
103. Kligman, A. M., and Ginsberg, D., *J. Invest. Dermatol.*, **14**, 345–58 (1950)
104. Wyckoff, R. W. G., *Nature*, **168**, 651–52 (1951)
105. Coons, A. H., Snyder, J. C., Cheever, F. S., and Murray, E. S., *J. Exptl. Med.*, **91**, 31–38 (1950)
106. Robertson, O. H., and Uhley, C. G., *J. Clin. Invest.*, **15**, 115–30 (1936)
107. Gunn, F. D., and Nungester, W. J., *Arch. Pathol.*, **21**, 813–30 (1936)
108. Bull, C. G., *J. Exptl. Med.*, **22**, 475–83 (1915)
109. Melnick, J. L., and Godman, G. C., *J. Exptl. Med.*, **93**, 247–66 (1951)
110. Loosli, C. G., *J. Infectious Diseases*, **84**, 153–68 (1949)
111. Chu, T. H., Cheever, F. S., Coons, A. H., and Daniels, J. B., *Proc. Soc. Exptl. Biol. Med.*, **76**, 571–74 (1951)
112. Taliaferro, W. H., and Cannon, P. R., *J. Infectious Diseases*, **59**, 72–125 (1936)
113. Cannon, P. R., and McClelland, P. H., *Arch. Pathol.*, **7**, 787–800 (1929)
114. Zuckerman, A., and Yoeli, M., *J. Infectious Diseases*, **89**, 130–42 (1951)
115. Taliaferro, W. H., Cannon, P. R., and Goodloe, S., *Am. J. Hyg.*, **14**, 1–37 (1931)
116. King, H., and Shumacker, H. B., Jr., *Ann. Surg.*, **136**, 239–42 (1952)
117. Miller, B. F., Abrams, R., Dorfman, A., and Klein, M., *Science*, **96**, 428–30 (1942)

118. Bloom, W. L., Watson, D. W., Cromartie, W. J., and Freed, M., *J. Infectious Diseases*, **80**, 41–52 (1947)
119. Bloom, W. L., and Blake, F. G., *J. Infectious Diseases*, **83**, 116–23 (1948)
120. Whitney, D. M., Anigstein, L., and Micks, D. W., *Proc. Soc. Exptl. Biol. Med.*, **74**, 346–50 (1950)
121. Nutini, L. G., and Lynch, E. M., *J. Exptl. Med.*, **84**, 247–61 (1946)
122. Bloom, W. L., Hudgins, P. C., and Cummings, M. M., *J. Infectious Diseases*, **92**, 70–73 (1953)
123. Björnesjö, K. B., *Acta Chem. Scand.*, **5**, 1412–13 (1951)
124. Hirsch, J. G., and Dubos, R., *J. Exptl. Med.*, **95**, 191–208 (1952)
125. Hirsch, J. G., *J. Exptl. Med.*, **97**, 327–43 (1953)
126. Dubos, R. J., and Hirsch, J. G., *J. Exptl. Med.*, **99**, 55–63 (1954)
127. Hirsch, J. G., and Dubos, R. J., *J. Exptl. Med.*, **99**, 65–78 (1954)
128. Synge, R. L. M., *Quart. Rev. Chem. Soc.*, **3**, 245–62 (1949)
129. Henley, D. A., *An Investigation of the Pneumococcidal Activity of Extracts of Tissues from Resistant and Susceptible Host Species* (Doctoral thesis, Univ. of Michigan, Ann Arbor, Mich., 1953)
130. Clark, E. C., Lathe, G. H., and Ruthven, C. R. J., *Brit. J. Exptl. Pathol.*, **34**, 542–47 (1953)
131. Duran-Reynals, F., and Duran-Reynals, M. L., *Science*, **115**, 40–41 (1952)
132. McCrea, J. F., and Duran-Reynals, F., *Science*, **118**, 93–95 (1953)
133. Bacon, G. A., Burrows, T. W., and Yates, M., *Brit. J. Exptl. Pathol.*, **32**, 85–96 (1951)
134. Goodlow, R. J., Braun, W., and Mika, L. A., *Arch. Biochem.*, **30**, 402–6 (1951)
135. Mika, L. A., Goodlow, R. J., and Braun, W., *Bact. Proc.*, 77–78 (1952)
136. Ackermann, W. W., *J. Exptl. Med.*, **93**, 337–43 (1951)
137. Thompson, R. L., and Lavender, A. R., *Federation Proc.*, **12**, 463 (1953)
138. Shaw, E., *Metabolism, Clin. and Exptl.*, **2**, 103–19 (1953)
139. Boivin, A., and Delaunay, A., *Presse méd.*, **53**, 217–18 (1945)
140. Marshall, A. H. E., *J. Pathol. Bact.*, **65**, 29–48 (1953)
141. Berry, L. J., and Spies, T. D., *Medicine*, **28**, 239–300 (1949)
142. Kerby, G. P., Holland, B. C., and Martin, S. P., *J. Immunol.*, **64**, 123–29 (1950)
143. Nungester, W. J., and Watrous, R. M., *Proc. Soc. Exptl. Biol. Med.*, **31**, 901–5 (1934)
144. Alvarez, D. A., Jr., *Am. J. Hyg.*, **56**, 31–38 (1952)
145. Boand, A. V., Kempf, J. E., and Hanson, R. J., *Federation Proc.*, **12**, 436 (1953)
146. Eisler, D. M., and Ecker, E. E., *Science*, **112**, 9–11 (1950)
147. Martin, S. P., and Kerby, G. P., *J. Exptl. Med.*, **92**, 45–49 (1950)
148. Kerby, G. P., and Martin, S. P., *J. Exptl. Med.*, **93**, 189–95 (1951)
149. Altschul, R., and Friesen, A. M., *Am. J. Clin. Pathol.*, **17**, 444–48 (1947)
150. Menkin, V., *Newer Concepts of Inflammation* (Charles C Thomas, Springfield, Ill., 145 pp., 1950)
151. Kline, D. L., and Cliffton, E. E., *Science*, **115**, 9–11 (1952)
152. Rosenow, G., *Acta Haematol.*, **5**, 1–18 (1951)
153. Wood, W. B., Jr., and Smith, M. R., *J. Lab. Clin. Med.*, **32**, 1406–7 (1947)
154. McCutcheon, M., *Physiol. Revs.*, **26**, 319–36 (1946)
155. Harris, H., *Brit. J. Exptl. Pathol.*, **34**, 276–79 (1953)
156. Meier, R., and Schär, B., *Experientia*, **9**, 93–94 (1953)
157. Martin, S. P., and Chaudhuri, S. N., *Proc. Soc. Exptl. Biol. Med.*, **81**, 286–88 (1952)

158. Martin, S. P., Pierce, C. H., Middlebrook, G., and Dubos, R. J., *J. Exptl. Med.*, **91**, 381–92 (1950)
159. Elberg, S. S., and Schneider, P., *J. Infectious Diseases*, **93**, 36–42 (1953)
160. Klepser, R. G., and Nungester, W. J., *J. Infectious Diseases*, **65**, 196–99 (1939)
161. Cromartie, W. J., Watson, D. W., Bloom, W. L., and Heckly, R., *J. Infectious Diseases*, **80**, 14–27 (1947)
162. Knisely, M. H., Bloch, E. H., and Warner, L., *Kgl. Danske Videnskab. Selskab Biol. Skrifter*, **7**(4), 5–93 (1948)
163. Felton, L. D., and Bailey, G. H., *J. Infectious Diseases*, **38**, 131–44 (1926)
164. Merchant, D. J., and Morgan, H. R., *Proc. Soc. Exptl. Biol. Med.*, **74**, 651–53 (1950)
165. Wood, H. F., *Proc. Soc. Exptl. Biol. Med.*, **76**, 843–47 (1951)
166. Smith, H., Zwartouw, H. T., Gallop, R. C., and Harris-Smith, P. W., *Biochem. J. (London)*, **53**, 673–78 (1953)
167. Ludány, G., Orbán, T., and Vajda, J., *Arch. intern. pharmacodynamie*, **88**, 496–502 (1952)
168. Smith, M. R., Perry, W. D., Berry, J. W., and Wood, W. B., Jr., *J. Immunol.*, **67**, 71–74 (1951)
169. Lerner, E. M., II, and Victor, J., *Proc. Soc. Exptl. Biol. Med.*, **80**, 110–12 (1952)
170. Steffee, C. H., *J. Infectious Diseases*, **86**, 12–26 (1950)
171. Kuna, A., Blattberg, B., and Reiman, J., *Proc. Soc. Exptl. Biol. Med.*, **77**, 510–14 (1951)
172. Cottingham, E., and Mills, C. A., *J. Immunol.*, **47**, 493–502 (1943)
173. Nungester, W. J., and Ames, A. M., *J. Infectious Diseases*, **83**, 50–54 (1948)
174. Bacchus, H., and Toompas, C. A., *Science*, **113**, 269–70 (1951)
175. Gell, P. G. H., and Hinde, I. T., *Brit. J. Exptl. Pathol.*, **34**, 273–75 (1953)
176. Marcus, S., Hill, G. A., and Esplin, D. W., *Proc. Soc. Exptl. Biol., Med.*, **84**, 565–68 (1953)
177. Lerner, E. M., II, and Victor, J., *Proc. Soc. Exptl. Biol. Med.*, **82**, 414–16 (1953)
178. Nungester, W. J., and Ames, A. M., *J. Infectious Diseases*, **90**, 51–60 (1952)
179. Ludány, G., Vajda, J., and Erdos, E., *Arch. intern. pharmacodynamie*, **87**, 49–63 (1951)
180. Timiras, P. S., *Acta Endocrinol., Suppl. No. 11*, 1–118 (1953)
181. Nungester, W. J., Ames, A. M., and Lanning, W., *J. Infectious Diseases*, 90, 61–66 (1952)
182. Fenn, W. O., *The Newer Knowledge of Bacteriology and Immunology*, 861–69 (The University of Chicago Press, Chicago, Ill., 1196 pp., 1928)
183. Fritze, E., *Acta Haematol.*, **4**, 351–57 (1950)
184. Rebuck, J. W., *Am. J. Clin. Pathol.*, **17**, 614–30 (1947)
185. Barnes, J. M., *Brit. J. Exptl. Pathol.*, **21**, 264–75 (1940)
186. Valentine, W. N., *Blood*, **6**, 845–54 (1951)
187. McKinney, G. R., Martin, S. P., Rundles, R. W., and Green, R., *J. Appl. Physiol.*, **5**, 335–40 (1953)
188. Rossiter, R. J., and Wong, E., *Can. J. Research*, **28**, 67–79 (1950)
189. Follette, J. H., Valentine, W. N., and Lawrence, J. S., *J. Lab. Clin. Med.*, **40**, 825–40 (1952)
190. Grogg, E., and Pearse, A. G. E., *Brit. J. Exptl. Pathol.*, **33**, 567–76 (1952)
191. Wong, E., *Federation Proc.*, **10**, 271–72 (1951)
192. Ackermann, W. W., and Francis, T., Jr., *Proc. Soc. Exptl. Biol. Med.*, **74**, 123–26 (1950)

193. Fieldsteel, A. H., and Preston, W. S., *J. Infectious Diseases*, **93**, 236–42 (1953)
194. Chaudhuri, S. N., and Martin, S. P., *J. Exptl. Med.*, **98**, 99–105 (1953)
195. Moulder, J. W., and Weiss, E., *J. Infectious Diseases*, **88**, 77–80 (1950)
196. Vogel, F. S., *J. Exptl. Med.*, **93**, 305–11 (1951)
197. Alonso, D., *A Comparative Study of the Metabolism of Leucocytes from Guinea Pigs and Rats* (Doctoral thesis, Univ. of Michigan, Ann Arbor, Mich., 1953)
198. Murayama, M., *A Study of the Adenosinetriphosphatase Activity of Leucocyte Nuclei Free Homogenates of the Guinea Pig and the Rat* (Doctoral thesis, Univ. of Michigan, Ann Arbor, Mich., 1953)
199. Berry, L. J., and Mitchell, R. B., *J. Infectious Diseases*, **93**, 75–82 (1953)
200. Berry, L. J., and Mitchell, R. B., *J. Infectious Diseases*, **93**, 83–92 (1953)
201. Rogers, D. E. and Tompsett, R., *J. Exptl. Med.*, **95**, 209–30 (1952)
202. Braude, A. I., and Feltes, J., *J. Lab. Clin. Med.*, **42**, 289–98 (1953)
203. Suter, E., *Am. Rev. Tuberc.*, **65**, 775–76 (1952)
204. Wilson, A. T., *J. Exptl. Med.*, **98**, 305–10 (1953)

EFFECTS OF VIRUSES ON TUMORS[1]

By Alice E. Moore

Virus Study Section, Sloan Kettering Institute of Memorial Center, New York, New York

Introduction

This review of the effects of viruses on tumors is divided into five sections: (*a*) solid animal tumors, (*b*) mouse leukemias, (*c*) ascites tumors of mice, (*d*) tumor cells in tissue culture, and (*e*) tumors in man. The viruses under each heading have been arranged alphabetically and without regard to chronological order.

The relationships between viruses and tumors have been considered from the following points of view: (*a*) the growth of viruses in tumor, (*b*) the effect of viral multiplication on tumor, (*c*) the effects of either tumor or virus, or both, on the host, and (*d*) any special feature of these relationships such as production of inclusion bodies, development of immunity, etc.

Effects of Viruses on Solid Animal Tumors

Avian pest.—Levaditi & Haber (1, 2) and Haber & Coquoin (3) discovered that the Schmidt strain of avian pest which was infective for mice had a definite affinity for transplantable mouse carcinomas (C-63 and T-146) and the Crocker sarcoma (sarcoma 180). Inoculated by any route, the virus caused a massive necrosis of the tumor which on transplantation into new hosts failed to grow. The tumor fragments carried sufficient virus with them to cause the death of the animals. Histologically, in addition to the necrosis, characteristic alterations in the nucleus and oxyphilic inclusions characteristic of the disease were found not only in the tumor but in the liver of the host.

When this same strain of virus was inoculated into the tumor or intravenously into rabbits carrying the Brown-Pearce tumor, it localized and multiplied in the neoplasm but had no effect on its growth or transplantability [Levaditi, Schoen & Reinié (4)]. Since these results contrasted sharply with those observed in mice, an attempt was made to adapt avian pest to rabbits by intrahepatic and intrasplenic passage. No pathogenicity for the rabbit developed, and the virus tended to die out. However, a strain which had been passed six times through liver and spleen still had an affinity for and multiplied in the Brown-Pearce tumor but had no inhibitory effect on tumor growth [Levaditi & Schoen (5)].

Coxsackie virus.—Levaditi & Henry-Eveno (6) found that strain B of Coxsackie virus failed to survive for more than four days when inoculated directly into the mouse epithelioma T-120 growing in adult mice. The virus failed to show any affinity for the tumor when inoculated intravenously, nor

[1] The survey of literature pertaining to this review was completed in January, 1954.

did it infect the tumor after *in vitro* exposure. No effect on tumor growth was noted.

Foot and mouth disease.—Bardach (7) reported that two types (O and A) of foot and mouth disease, when inoculated into the foot pad of a guinea pig, selectively localized in a transplantable liposarcoma and gained a high titer in the tumor tissue. Bridré & Bardach (8) with Rinjard (9) found that although guinea pigs could be immunized with the tumor grown virus, no immunity in cattle was produced until freshly infected tumor was added to their inactivated vaccine. They also found that the virus infected tumor was a satisfactory agent for producing hyper-immune serum in cattle [Rinjard, Bardach & Bridré (10)].

Herpes simplex.—Levaditi & Nicolau (11) inoculated herpes virus (White strain) into a transplantable mouse epithelioma and found that it multiplied there and had no effect on the growth of the tumor. The authors mention that the virus also grew in a mouse sarcoma. However, Levaditi & Schoen found (12) that herpes virus inoculated intravenously or directly into the Brown-Pearce carcinoma in rabbits failed to parasitize the tumor. In a study of the effect of herpes simplex virus on sarcoma 180, Moore (13) stated that although the virus persisted in the sarcoma 180 and could be carried with the tumor for four successive generations, it showed no preference for the tumor tissue when inoculated intracerebrally or intraperitoneally. It failed to multiply in a transplantable adenocarcinoma (EO771) and did not parasitize either tumor when exposed *in vitro*. No effect on tumor growth was noted.

Levaditi & Nicolau (14) were surprised to find what they considered to be an "activation" effect of herpes on vaccinia. An epithelial tumor which had been inoculated with vaccinia was later inoculated with herpes and five days later was found to contain only vaccinia virus.

Influenza.—Moore (13) found that influenza A injected into the sarcoma 180 in mice could be recovered for three days but had no effect on tumor growth and was not carried with the tumor on transplantation. No viral infection could be established in the carcinoma EO771, nor was it possible to infect either of these tumors *in vitro*.

Latent viruses.—Kritzler, Mulliken & Turner (15) have characterized an unknown virus which was isolated from a transplantable mouse carcinoma EO771 in C57 black mice as a passenger virus. It produced inclusions in the tumor and had no effect on its growth. It was also found in the tissues of C57 black mice which showed neutralizing antibodies to the agent. It was passed serially in eggs and produced skin lesions in Swiss mice.

Another example of a latent virus will be found under the discussion of Virus III.

Neurotropic virus survey.—Koprowski & Norton (16) have surveyed a large number of neurotropic viruses for their ability to inhibit the growth of five transplantable mouse tumors. It was found that West Nile was effective in inhibiting the growth of a methylcholanthrene-induced sarcoma (MCI),

EO771 carcinoma, and Wagnar and Ridgway osteogenic sarcomas, and was only partially effective against the sarcoma 180. Louping ill, St. Louis, and Ilhéus had an oncolytic effect on the MCI sarcoma, sarcoma 180, and the carcinoma EO771, whereas Bunyamwera virus affected only the two osteogenic sarcomas. The results with Venezuelan encephalitis virus were in general equivocal, but the virus appeared to display an oncolytic effect against the two osteogenic sarcomas. It seemed to inhibit the growth of the originally implanted EO771 carcinoma and MCI tumor but had little effect on their transplants. Several viruses, for example Japanese B, Semliki, and Bunyamwera, attained very high titers in the MCI and EO771 tumors but had no effect on their growth. The behavior of Eastern equine encephalitis was paradoxical in that it failed to multiply in the rapidly-growing sarcoma 180, yet attained a high concentration in the slower-growing Ridgway osteogenic sarcoma but did not influence tumor growth.

The following had no effect on the growth of the two sarcomas, two osteogenic sarcomas, and one adenocarcinoma: French neurotropic yellow fever, Bwamba, Ntaya, Passos I, pseudorabies, Semliki, Japanese B, Western, and Eastern equine encephalitis. It was observed that in some instances it is difficult to infect the tumor-bearing mice by peripheral routes so that the tests may not be absolutely conclusive.

Sharpless, Davies & Cox (17) have surveyed numerous viruses for ability to inhibit a lymphoid chicken tumor RPL12. Their positive results with Russian encephalitis and St. Louis will be recorded. In addition, West Nile and Japanese B viruses were capable of inhibiting tumor growth. Negative results were reported with the following viruses and rickettsia: mumps, swine influenza, canine distemper, Columbia S-K, Bwamba, Semliki Forest, Ilhéus, GD VII, Eastern equine encephalitis, Western equine encephalitis, three strains of rabies, Rocky Mountain spotted fever, North Queensland tick typhus, rickettsial pox, boutonneuse fever, South African tick fever, epidemic typhus, murine typhus, lymphogranuloma venereum, feline pneumonitis, human pneumonitis, and bovine encephalitis.

Pox viruses.—Ectromelia: Schoen (18) studied the effect of ectromelia on the Ehrlich carcinoma and an epithelial tumor which has arisen as a result of painting with tar. She found that virus inoculated into the tumor, intracerebrally or intraperitoneally, localized in the tumor causing its complete destruction and failure to subtransplant. Inclusions of the same type described for the liver were found in the tumor tissue. Exposure *in vitro* of cancer tissue to the virus for 2 hr. at room temperature resulted in complete inhibition of tumor growth. All mice succumbed to a generalized infection.

Vaccinia: This virus was the first to be studied in relation to transplantable animal tumors. Levaditi & Nicolau had developed the theory that tissue affinities of viruses were dependent on the germ layer from which the tissue arose (19). The fact that neurovaccinia proliferated well in transplantable epitheliomas of mice and rats but poorly, if at all, in sarcomas of these animals was taken as evidence for the theory [Levaditi & Nicolau (20,

21, 22)]. The virus, after displaying an initial stimulatory effect on tumor growth, caused necrosis, and a few cures were reported. On transplantation the virus-bearing tumors grew poorly if at all. When tumors did grow they were as susceptible to the virus as had been their parent. It was noted by these same authors that an epithelioma implanted into a neurovaccinia immune rat grew normally and failed to support viral multiplication; but when this tumor was transplanted to nonimmune hosts, it was again susceptible. A difference between the behavior of normal and neoplastic tissue was commented on by noting that susceptible tissue, such as skin and cornea, after recovery from infection was immune to reinoculation, in contrast to malignant tissue which continued to support viral growth. Tumors were found to be poor "selectors" of neurotropic variants of dermovaccinia when compared with intracerebral passage in rabbits or allantoic membrane passage in eggs [Levaditi (23)].

Levaditi & Nicolau (19, 24), in a study of the effect of dermo- and neurovaccinia on the contagious epithelioma of birds (fowl pox), found that dermovaccinia proliferated in much the same way as it did in epitheliomas of rats and mice and that it persisted for long periods, even in vaccinia-immune birds. Neurovaccinia, which under usual circumstances failed to grow in the cock's comb, grew and persisted in the neoplastic lesion, when inoculated simultaneously with the fowl pox.

In rabbits bearing the Shope papilloma, Levaditi & Schoen (25) found that inocultion of neurovaccinia by intracutaneous, intravenous, or intratumoral routes resulted in the appearance of large quantities of virus in the tumor. Guanieri bodies were also found in the tumor. In this same species Rivers & Pearce (26) noted that a transplantable epithelioma (Brown-Pearce tumor) inoculated with vaccinia carried the virus with it when transplanted and that such tumors conferred vaccinial imunity on its host. The virus survived longer in the tumor than in other tissues.

More recently Turner & Mulliken (27, 28) showed that neurovaccinia inoculated directly into sarcoma 180 or by the intravenous route multiplied in the tumor. Inhibition of growth of the tumor after direct inoculation was indicated by failure of transplants to grow as successfully as the uninoculated controls. No signs of vaccinial infection were noted in the mice. Histological studies of vaccinia-infected sarcoma 180 by Kritzler, Mulliken & Turner (29) showed no definite difference between controls and vaccinia-infected tumors except for a decrease in the mitotic figure count in the latter. A different effect was noted by Taylor & Carmichael (30) who found that a dba tumor growing in the yolk sac of the developing egg protected the embryo from death when vaccinia was inoculated into the yolk. A C3H mammary tumor and a rat sarcoma were without effect.

Psittacosis-Lymphogranuloma Viruses.—Lymphogranuloma: Schoen (31, 32, 33) showed that mouse-adapted lymphogranuloma virus (Kam strain) grew well in the Ehrlich sarcoma and produced characteristic inclusion bodies. The tumor whose growth was unaffected by the virus, could be used

as a source of Frei antigen. The virus could be maintained in serial passage and retained its infectivity longer in tumor tissue than in normal tissue. In two instances, where spontaneous carcinomas arose in animals which had been used for lymphogranuloma experiments 57 and 90 days previously, virus was found in the neoplasms, although little was found in normal tissue [Schoen (34)]. Schoen (35) also found that although the virus multiplied in a tar-induced epithelioma, it usually failed to produce inclusion bodies and barely survived in another transplantable mouse epithelioma, C63. It was concluded that this virus had a selective affinity for tumors of mesodermal origin.

Ornithosis: Kuwata (36) reported that the Kam strain of ornithosis, while capable of multiplying in the fructose-induced sarcoma and the quinone-induced carcinoma, had only a partial inhibitory effect on the growth of the tumors.

Rabies.—Levaditi & Schoen (37) and Levaditi, Schoen & Reinié (38) studied street rabies virus (Bucharest II strain) in the Brown-Pearce carcinoma of rabbits. They found that virus inoculated intracerebrally or into either eye multiplied and produced Negri bodies in tumors growing in the anterior chamber of the eye. Negri bodies were also found in the brain tumors which resulted from intracerebral inoculation of the virus-infected tumor. It was possible to pass the virus-infected intraocular tumors in series, although the virus seemed to have a somewhat adverse effect on tumor growth.

When the tumors containing virus were implanted subcutaneously or in the testicle, they produced rabies in the rabbit; but on the rare occasions when tumors grew at the implantation site, they contained no virus. Similarly, tumor fragments exposed to virus *in vitro* also conferred rabies on their host but did not contain virus or Negri bodies when they grew. Metastatic tumors or subcutaneously-implanted tumors failed to become infected when the virus was inoculated intracerebrally (38). The authors concluded that for the complete cycle of development of the rabies virus with the production of Negri bodies the tumor must grow either in the brain or the anterior chamber of the eye.

Two strains of fixed rabies virus (Pasteur and Tunis) grew with difficulty in tumors in the anterior chamber of the eye, and serial passage of the virus was impossible (38). Schoen (39) found that freshly-isolated strains of street rabies virus were capable of growing in intraocular Brown-Pearce carcinomas but produced no Negri bodies unless transplants were made to the brain.

In a study of street rabies infection of the Shope papilloma, Levaditi, Schoen & Reinié (40) noted that either intraocularly or subcutaneously implanted tumors failed to support or weakly supported viral multiplication, although the animals died of the infection. Haguenau, Cruveilheir & Viala (41) found that this same tumor failed to develop in lactating animals which were given a series of rabies inoculations. The development of the tumor was not affected by the rabies alone or by the lactating state alone. Three strains of fixed rabies virus (Pitman Moore No. 980, Flury, and Novisad)

were found to have no effect on the chicken tumor RPL12 (17).

Rickettsia tsutsugamushi.—Kuwata (36) observed that the AG ANO-X strain of rickettsia injected directly into a fructose-induced sarcoma or quinone-induced carcinoma attained a higher titer than in the liver for the first week of infection. Bioassays from the heavily-infected tumors failed to grow, and the mice usually died of the infection.

Rous sarcoma.—Mellanby (42), in a study of the dissemination of Rous sarcoma virus I to two nonfilterable tumors of chickens (one dibenzanthracene-induced, the other from tar), found that when these two and a Rous tumor were present in the same animal, a cell suspension from the nonfilterable tumor resulted in production of a similar tumor in a new host, but that a cell-free filtrate of the same tumor often resulted in the production of the Rous sarcoma. Although the Rous sarcoma virus occasionally appeared in the second and third generation of the nonfilterable tumors, it was unusual. In two instances regression of the dibenzanthracene tumor was followed by the appearance of Rous sarcomas. This was interpreted as being attributable to latent Rous virus rather than as being a direct effect of the virus on the tumor.

In pursuing further the relationship between filterable and nonfilterable tumors, Carr (43) found that two nonfilterable chicken tumors, GRCH/15 and Mca 1, only occasionally showed Rous virus when they were inoculated into the same host.

Russian far east encephalitis.—Moore (44) and Moore & O'Connor (45) have shown that the virus of Russian encephalitis destroyed the transplantable mouse sarcoma 180 regardless of the route of inoculation of the mouse or the size of the tumor. The length of time necessary for complete destruction depended on the amount of virus inoculated and the presence of active infection in a nonimmune host. Microscopic examination of the affected tumors showed pyknotic nuclei, cellular fragmentation, and necrosis, but no specific changes. Comparative titrations showed that the virus appeared first and in the highest concentration in the tumor and remained at a level higher there than in any other tissue until the death of the animal. That a fatal encephalitis was not an essential feature of tumor destruction was demonstrated by the following experiment: Pieces of sarcoma 180 were implanted in a strain of mice (PRI) developed at the Rockefeller Institute in Princeton. These mice are genetically resistant to the lethal effects of Russian encephalitis. Intraperitoneal inoculation of virus propagated by passage in tumors resulted in disappearance of the tumor with no sign of illness in the mice [Moore (46)].

Long-continued serial passage of Russian encephalitis in tumor tissue resulted in an increase in the ability of the virus to destroy the tumor, while similar passage of the same virus in the brain resulted in a great loss of this ability. Passage in the Wagnar osteogenic sarcoma resulted in the virus becoming destructive for this hitherto nonsusceptible tumor. No change in neurotropism of these adapted viruses was noted [Moore (47)]. When dif-

ferent types of transplantable mouse tumors were studied it was found that they varied greatly in their susceptibilities to destruction by the virus. Some were destroyed readily by small amounts whereas growth of others was not affected at all [Moore (48)]. One, the Harding-Passey melanoma, failed to support viral multiplication (49).

Koprowski & Norton (16) in their survey of viral activity against transplantable tumors also found Russian encephalitis and Louping ill, which are antigenically similar, effective against four of five mouse tumors. Sharpless, Davies & Cox (17) were able to cause complete regresssion of the chicken tumor RPL12, with host survival in a high percentage of cases, by inoculation of Russian encephalitis into the contralateral breast. They observed high titers in the tumor tissue and noted that passage of virus in the tumor increased the oncolytic effect. Birds which had recovered from the tumor through virus inoculation were immune to reinoculation of the RPL12 tumor.

St. Louis encephalitis.—Koprowski & Norton (16) included St. Louis encephalitis in their survey and found it had an oncolytic effect on an MCI sarcoma and the adenocarcinoma EO771. The virus also caused complete regression of the chicken tumor RPL12 with survival of the host [Sharpless, Davies & Cox (17)]. Buckley, Buckley & Snipes (50) found that the virus attained higher titers in the sarcoma 180 than in other tissues for eight days after intraperitoneal inoculation, and as a result, tumor growth was less than that of the controls for the first two weeks. Histological examination showed less mitosis and more necrosis in the infected tumors.

Semliki Forest virus.—Ginder & Friedewald (51) found that Semliki Forest virus multiplied in tissue culture preparations made from rabbit fibromas, and when the Semliki Forest virus was inoculated with fibroma virus in rabbits, it completely suppressed tumor development. In tumors which had already developed, the virus caused premature regressions and multiplied until the tumor regressed. In a study of this virus and that of rabbit myxoma the same authors (52) found that when the two viruses were inoculate simultaneously, 8 of 21 animals survived as contrasted to 100 per cent fatalities in the controls. After 45 passages from tumor to tumor, the percentage of survivors from myxoma was increased, and some animals could be saved if the Semliki Forest virus was inoculated as late as one day after the myxoma virus. The virus persisted in the myxomatous lesions after it had disappeared from the blood and secretions.

Semliki Forest virus has been found to be ineffective in inhibiting the growth of the RPL12 chicken tumor [Sharpless, Davies & Cox (17)] and five transplantable mouse tumors, although it attained high titers in the tumor tissue [Koprowski & Norton (16)].

Virus III.—Rivers & Pearce (26) found that the Brown-Pearce carcinoma carried Virus III and conferred antiviral immunity on the host with transplantation. The virus had no effect on the growth of the neoplasm and could be separated from it. Young virus-carrying tumors contained inclusion

bodies and, when extracts were inoculated intradermally into rabbits, produced the characteristic skin reaction. It was also ascertained that the virus persisted longer in the tumors than in the testicle of a normal animal and could be detected in the tumor growing in an immune animal. Pearce & Rivers (53) studied the malignant disease produced by virus-free and virus-carrying tumors, and although the differences were not marked, the virus-carrying tumors seemed to produce a more severe disease. When virus-immune and susceptible rabbits were used for implantation of virus-free and virus-carrying tumor, the least malignant disease occurred in the virus-immune group which had been implanted with virus-carrying tumor (54). The authors considered these results to be attributable to a nonspecific cause, such as varied reaction on the part of the host.

Andrewes (55), too, found that during pasage his infectious fibroma of rabbits had become infected with Virus III and that this caused premature regressions of the fibroma. When the fibroma virus was inoculated and 4 hr. later Virus III was injected into the same spot, no fibroma appeared, although the virus had no such effect if inoculated at a distance. A transplantable rabbit sarcoma RSI, also found to be carrying the virus, was not affected by it. In both tumors characteristic inclusions were found, and in the rabbit fibroma central necrosis appeared in connection with the previously-mentioned premature regressions.

Yellow fever.—Findlay & MacCallum (56) found that the transplantable mouse sarcoma 37S failed to support yellow fever virus although the carcinoma 63 did. The virus caused premature necrosis of the tumors, but no characteristic cytological changes were noted. When inoculated intraperitoneally or subcutaneously, virus was found in the tumor long after it had disappeared from the blood. Of three strains serially passed in the carcinoma, one seemed to lose some of its pathogenicity for the monkey. Koprowski & Norton (16) found no effect of the French neurotropic strain of yellow fever on five transplantable mouse tumors.

Effects of Viruses on Mouse Leukemias

Ilhéus and West Nile.—Southam, Bronstein & Webber (57) studied the effect of Ilhéus and West Nile viruses on leukemia and found that there was a selective inhibition of leukemia blast cell proliferation in the AK4 leukemia and absence of hepatosplenomegaly. West Nile virus was found to localize in the spleen, and the Ilhéus virus could be shown to be located preferentially in the leukocytes, indicating a preference for malignant tissue. Similar results were found for the AK4R leukemia, an aminopterin-resistant variant of the parent AK4. In the 9421 leukemia, which kills more slowly, the results were not as striking, but the viruses did show an effect against both mature and immature lymphoid cells. In all instances the mice died of viral infection.

Latent virus.—MacDowell, Taylor & Broadfoot (58) describe a virus which had been associated with line I transplanted leukemia for many years.

When 15 leukemic cells of the virus-carrying strain were inoculated, all of 257 mice survived, in conrast to fatalities from leukemia in 51 of 52 mice inoculated with the same number of virus-free leukemic cells.

Russian encephalitis.—Moore & Stock (59) briefly noted that Russian encephalitis caused an inhibition of AK4 and 9421 leukemia as measured by size of spleens and livers. Southam & Epstein (60), in a study on the effect of this virus on AK4 leukemia, showed that the blast forms and leukocytes were specifically reduced in number even if the Russian encephalitis was injected as late as six days following the inoculation of the leukemia. A decrease in weight of spleen and liver was also noted when compared to the controls.

Survey of viruses.—Turner (28) noted that influenza A, Theiler's virus, and rickettsial pox had no effect on two leukemias. Southam & Epstein (60) observed that Bunyamwera, Uganda S, Anopheles B, Newcastle Disease (Massachusetts strain), and GD VII had a slight or inconsistent inhibitory effect, whereas Zika, Semliki, M. M. (encephalo-myocarditis virus), neuroinfluenza, dermovaccinia, neurovaccinia, Newcastle Disease (CG179), MacDowell's virus (see above), and Ntaya had no effect. The authors point out that many of these viruses, in cases where they might be expected to be active, may not have infected the animal when inoculated peripherally.

Vaccinia.—Turner & Mulliken (28) have reported 16 per cent survivors when a neutrotropic strain of vaccinia was inoculated intravenously into mice which had been injected with leukemia 9417, which regularly killed almost all mice in two to four weeks. Subsequently, the leukemia became more malignant and killed in a much shorter time. Under these conditions the inhibitory effect of the virus disappeared. The course of the disease produced by two other strains of leukemia, 1394 and C1498, was unaffected by neurovaccinia.

Effect of Viruses on Ascites Tumors of Mice

The ascites tumors of mice offer many advantages in the study of tumor cell-virus relationships. These have recently been summarized by Koprowski, Koprowska & Love (61).

Viruses Apparently Not Multiplying in Cells.—Hemmagglutinating viruses: Kausche, Landschutz & Sauthoff (62) noted that the injection of mixtures of Ehrlich carcinoma ascitic fluid and influenza virus failed to result in ascites formation. If tumor cells were injected first and virus afterwards, no inhibiting influence was noted. Moore & Diamond (63, 64), and Moore *et al.* (65), working with Massachusetts and CG179 strains of Newcastle Disease virus and two types of ascites tumors, Ehrlich carcinoma and sarcoma 180, noted similar results. These investigators found that tumor cells mixed with either Newcastle Disease or any of four strains of influenza virus failed to grow when inoculated immediately after mixing at 4°C., 25°C., or 37°C. After mixing, a fall in hemagglutinating activity and infectivity of the supernatant occurred, indicating adsorption of the virus to the cell. With the two strains of Newcastle Disease mentioned it was noted that following ad-

sorption at 4°C. or 25°, tumor cells in the mixture regained their ability to grow if left for from 1 to 2 hr. at room temperature. This was accompanied by a rise in hemagglutinin and infectivity in the supernatant fluids. Since it was impossible to demonstrate the multiplication of Newcastle Disease virus in the tumor cells *in vivo* or in tissue culture, the authors have considered this to be a phenomenon similar to that of the adsorption and elution of virus to and from red blood cells. The inhibition of tumor growth which took place with the two strains of Newcastle Disease virus and the four strains of influenza (PR8, LEE, WS, and Swine) was dependent on the amount of virus and the number of cells in the mixtures. Elution took place only with the Newcastle Disease virus, and following elution the cells were no longer capable of being inhibited when fresh virus was added. Newcastle Disease virus inactivated by heat, ultraviolet irradiation, ultrasonic vibration or formalin readily lost its ability to inhibit tumor cells; but the irradiated virus was capable of blocking the inhibitory effect when active virus was added. Tumor cells treated with receptor destroying enzyme (RDE) were incapable of being affected by any of the viruses studied and if RDE were added to the adsorbed tumor-virus mixture, it removed the virus with resulting recovery of growth ability of the cells. Virus could also be removed by specific immune serum. Price & Ginsberg (66) in *in vivo* experiments, noted the inhibitory effect of Newcastle Disease virus on the Ehrlich ascitic tumor and found that RDE-treated cells failed to be affected by the virus. They also found that cells treated with ultraviolet were not affected by the addition of active virus. Anti-viral serum administrered intraperitoneally before the virus neutralized the tumor inhibitory effect, but was ineffective if given 15 min. after the virus.

Viruses multiplying in ascites tumor cells.—Bunyamwera virus: Koprowska & Koprowski (67) found that Bunyamwera was the most effective agent of all those which they tested for oncolytic ability. Love, Koprowski & Cox (68) made a cytological study of Ehrlich ascites cells before and after infection with Bunyamwera virus. They found a sharp drop in the percentage of malignant cells and in mitotic figures within two to three days and an increase in the percentage of degenerating cells. A detailed account of the cytopathological changes is given by these authors.

Influenza: Ackermann & Kurtz (69) adapted the WS strain of neurotropic influenza to the Ehrlich ascites tumor and were thus able to study the growth characteristics of the virus. They found that the virus inhibited the development of ascites.

Neurotropic virus survey: Koprowska & Koprowski (70) were the first to call attention to the advantages of studying the growth of viruses in the ascitic tumors of mice. They surveyed (67) the effects of 12 viruses for (*a*) ability to grow, (*b*) effect of viral infection on the growth of the Ehrlich ascites tumor, and (*c*) cytopathogenic effects on the cells. The following viruses failed to grow and thus had no effect on the tumor: Eastern equine encephalitis, Western equine encephalitis, Semliki Forest virus, and rabies.

The following seemed to multiply in the tumor and sometimes had a mild inhibitory effect: Japanese B, GD VII, St. Louis, Louping ill, neuroinfluenza, and Ilhéus. West Nile virus (two strains) was unique in the fact that it prevented cell growth on bioassay but induced no cytopathologic damage.

Mengo encephalitis: In a study of the effect of Mengo virus on the Ehrlich ascites tumor, Koprowska & Koprowski (71) found that the virus attained a titer one to two logs higher in ascitic than in normal mice. The marked oncolytic effect produced by the virus was accompanied by an increase in percentage of degenerating cells, a decrease in the number of malignant cells to almost nothing, and complete suppression of mitosis.

St. Louis encephalitis: Cheever & Dickos (72) found that the maximum titer of St. Louis encephalitis in Ehrlich ascites cells was attained in the group inoculated with tumor four days previously. After 14 passages in the ascitic tumor the titer in cells had increased one log. A slight oncolytic effect was also noted.

Effect of Viruses in Tissue Culture

Before more sophisticated methods were developed, viruses were grown in a Maitland type tissue culture which consisted of minced viable tissue and a balanced salt solution. Mouse tumors (37S sarcoma and carcinoma M63) were used as a source of viable cells by Findlay & MacCallum (73) for the growth of yellow fever virus in this type of tissue culture medium. Hallauer (74) reported that virus of avian pest could propagate in cultures of minced mouse tumors. Harrison & Julianelle (75) failed in their efforts to grow trachoma virus in cultures of minced Brown-Pearce carcinoma.

Eastern equine encephalitis.— Direct observation of the effect of viruses on tumor cells grown in tissue culture was initiated by Bang & Gey (76, 77), who observed that Eastern equine encephalitis selectively destroyed the cells of a rat sarcoma T333 and had no effect on the parent fibroblast 14p from which the malignant derivative arose. As the authors point out (78), this effect did not depend on the fact that this strain of cells was malignant, since another strain of rat fibroblast was also destroyed. They also noted that a human chondro-myxosarcoma (D-1 Re) was completely destroyed, and a human fibrosarcoma (A Fi) was partially destroyed, by Eastern equine encephalitis. Cells of the Walker rat carcinoma 256 were classified as being of intermediate susceptibility in that they were partially destroyed, but host cell recovery provided new cells for continuous virus survival. The authors (78) also noted the focal destruction of rat sarcoma cells T333 by small amounts of Eastern equine encephalitis and pointed out that this might be used as a method of titrating virus.

Herpes simplex.—Scherer (79) demonstrated that herpes simplex virus multiplied in L strain cells and produced inclusions in some of them. [L strain cells (Earle) produce sarcomas when inoculated into mice.]

Influenza.—DeLustig & Parodi (80, 81) state that cells of a mouse fibrosarcoma showed some cytological changes attributable to influenza A

virus, although these cells were much more resistant than cells derived from chicks. A mouse adenocarcinoma was even more resistant to action of the virus.

Poliomyelitis.—Scherer, Syverton & Gey (82) have reported the oncolytic effect exerted by the three types of poliomyelitis virus on a tissue culture strain of epithelial cells originally derived from a carcinoma of the cervix (HeLa). High titers were obtained and the destructive effect could be neutralized by specific immune serum. Stoler & M. Gey (83) also noted the destruction of a human fibrosarcoma (A Fi) by all three strains of poliomyelitis and neutralization of the cytopathogenic effects by specific sera.

Pseudorabies.—Scherer (79) found that the virus of pseudorabies readily multiplied in cultures of L cells (Earle). Characteristic intranuclear inclusions were also found.

Vaccinia.—Although vaccinia did not multiply in L cells, it was capable of causing agglutination of tissue culture preparations [Scherer (84)]. The effect could be neutralized by specific immune serum and did not take place with either the influenza or mumps viruses.

Virus survey.—Pollard & Bussel (85) studied the effect of eight viruses on various animal and human tumors grown in tissue culture. They found that a rat and mouse sarcoma were destroyed by St. Louis encephalitis virus and that neuroinfluenza had a slight inhibitory effect upon these tumors whereas rabies seemed to stimulate cellular growth. Newcastle Disease virus, mumps, influenza A, herpes simplex, and a strain of Lansing poliomyelitis had no effect. An undifferentiated human parotid tumor and a human anaplastic adenocarcinoma also were destroyed by St. Louis encephalitis, whereas Newcastle Disease had no effect and rabies seemed to have stimulatory activity for the adenocarcinoma.

West Nile Virus (Egypt 101 strain).—Toolan & Moore (86) showed that a human epidermoid carcinoma grown in irradiated rats and simultaneously in tissue culture was not only a favorable medium for Egypt 101 multiplication but that the tumor cells were completely destroyed in the animal host and in tissue culture.

The Effect of Virus Infection on Tumors in Man

The clinical literaure contains many references to beneficial effects of bacterial or viral infections on the progress of malignant disease. As early as 1912, DePace (87) noted an unexpected improvement in a case of cancer of the cervix following vaccination for rabies. Salmon & Baix (88) noted that vaccinia virus grew and produced characteristic lesions in cancerous tissue of the breast. These accidental observations and the fact that some viruses have a deleterious effect on the growth of animal tumors have led to the deliberate infection of man with these agents.

Bunyamwera.—Southam & Moore (89) reported the production of a severe case of encephalitis with residual symptoms in a patient with leukemia. Three other patients, one of whom received the same virus prepara-

tion, failed to develop the infection. No antileukemic effect was noted.

Dengue.—Erf (90) has reported that five patients with leukemia failed to show any benefit from inoculation with dengue virus.

Feline panleukopenia.—Bierman and co-workers (91) inoculated four children suffering from acute leukemia with the virus of feline panleukopenia. One had a clinical and hematological remission but no effect was noted in the others.

Hepatitis.—Hoster, Zanes & von Haam (92) reported amelioration in seven of thirteen patients with Hodgkin's disease following artificially-induced viral hepatitis.

Ilhéus.—Southam & Moore (89) inoculated Ilhéus virus into 19 patients with far advanced neoplasms. Nine developed the infection, and three had symptoms of a mild encephalitis. Localization of virus in the tumors occurred in four patients, but no inhibition of tumor growth could be demonstrated.

Newcastle disease.—Nine patients with advanced cancer inoculated with Newcastle Disease virus showed no clinical reaction other than fever and nausea. No effect on the neoplasms was noted [Southam & Moore (93)].

Rabies.—Following the observation of what appeared to be a regression of cancer of the cervix after rabies vaccination, DePace (87) inoculated eight other patients. Of four which could be evaluated, one was much improved, two were improved, and one obtained no benefit. Higgins & Pack (94) and Pack (95) have commented on their experiences in using rabies vaccine (Harris) in an effort to change the course of melanomas. Of 30 patients, 8 showed some type of regression of a temporary nature. In one instance, a metastatic nodule in the liver decreased sufficiently in size for surgical removal.

Rickettsial Pox.—Erf (90) inoculated rickettsial pox into two persons with leukemia without any beneficial effect.

Vaccinia.—Salmon & Baix (88) inoculated vaccinia virus into the nodules of carcinomatous tissue of a breast adenocarcinoma and noted that it proliferated and produced characteristic lesions. No viral spread to axillary nodes was noted, nor was any beneficial effect observed. Blank (96), in a review of virus diseases of the skin, mentioned inoculation of vaccinia into warts. The attempt had to be abandoned, however, because of systemic reactions. Southam & Moore (93) reported one case in which vaccinia was inoculated in an individual with advanced carcinoma, but no infection occurred.

Varicella.—Bierman *et al.* (91) noted a temporary remission associated with chicken pox in two cases of acute leukemia in children. In one of the cases a septicemia was a complication. One attempt to produce the disease artificially failed.

West Nile.—West Nile encephalitis virus strain M-956 isolated in 1937, and a recently isolated strain (Egypt 101), have been used extensively by Southam & Moore in clinical studies on many different types of far advanced

cancer. The M-956 strain (89), which had undergone many passages through mouse brain, caused a consistent viremia in only 5 of 21 patients inoculated and, although localization could be demonstrated in tumor tissue, no inhibitory effects were noted. The Egypt 101 strain, however, produced a significant viremia in 90 per cent of the cases. In a study of this virus in 34 patients it was found that viremia occurred in 27 and that the virus was found to localize specifically in approximately half of the tumors and to exert a transient inhibitory effect on tumor growth in at least four (93). In a later study the authors (97) reported on the effects of Egypt 101 inoculation in 95 patients, with special reference to infection, symptomatology, virus distribution, and the factors influencing the infection.

Koprowska (98) has attempted to evaluate the therapeutic effect of viral infection by examination of exfoliated cells from some of these patients. Although a decreased number of malignant cells was noted in two of six patients, she pointed out the difficulties inherent in depending on this method alone for clinical evaluation.

Comment

Since amost all investigators who have grown viruses in tumors have been seeking an answer to their particular problem, the facts which have emerged touch on many aspects of tumor-virus relationships. All can agree, however, that many viruses grow in tumors in many different species and that they may completely or partially inhibit tumor growth or may have no effect at all. The conditions under which tumor destruction takes place may be examined to see if there are any clues which will allow one to predict which combination of tumor and virus will result in oncolysis. These factors will be discussed in three categories: (*a*) the virus, (*b*) the tumor, and (*c*) the host.

The virus.—Since many different viruses have been shown to be effective in tumor destruction, the virus itself does not appear to be the governing factor. The virus does not have to produce a fatal infection, since there are many examples of tumor disappearance with host survival as well as of the death of the host with tumor survival. Although many of the most effective viruses for mouse tumors are neurotropic, their nerve tissue tropism cannot be the determining factor since other neurotropic viruses and even viruses which have been modified by passage in brain have little or no inhibitory effect on tumor growth. A true oncolytic effect as shown by cytological or biological evidence of tumor disintegration takes place only in the presence of active viral multiplication in the host and the tumor tissue; yet some viruses have been shown to prevent development of tumors when cells and virus are mixed *in vitro*. This has been interpreted as the result of the presence of virus on the cell surface, since viral multiplication has not been observed. In each instance where oncolysis has taken place, the virus has shown either a preference for the tumor tissue or it has multiplied to as high a titer in the tumor as in the most susceptible normal tissues.

The tumor.—When the tumor part of the virus-tumor relationship is

examined, it is obvious that the histological type, the rate of growth, or the site in the animal all fail to determine whether it will be destroyed or not. There are numerous examples of oncolysis in both transplantable and virus-induced tumors. The effects of viruses on spontaneous tumors have not been extensively studied in animals, and the only evidence for inhibitory effect on tumor growth is in man.

The host.—The host animal affects the outcome in that some viruses fail to grow in certain species and therefore have no oncolytic effect. Also, in most instances, if the host has been made immune, the virus is neutralized before oncolysis takes place. However, in a few cases where the virus is associated and transferred with a tumor, it persists in tumor tissue in an immune animal for long periods. The ideal host is an animal such as the PRI mouse whose natural resistance allows viral multiplication and tumor destruction with no perceptible illness of the animal.

It is obvious after a consideration of the factors which are involved in the growth of viruses in tumor that there is, as yet, no way of predicting which tumor will support viral multiplication or what the effect on the tumor cells will be.

One can only speculate on the mechanism involved in oncolysis, since so little is known about how an animal virus attacks any cell. Interference has been mentioned as one possible mechanism, and indeed it may play a role in a few specific instances, but the fact that there are no time relationships and that small amounts of virus can cause oncolysis makes this mechanism seem unlikely. It seems more probable that viruses cause cell destruction through disruption of intracellular metabolism by such mechanisms as competition for metabolites necessary for both cell and virus, or by the blocking of metabolic pathways by excessive accumulation of intracellular metabolites. If the virus interferes with tumor cell metabolism the cell may be killed; if not, both the virus and tumor may live in a symbiotic relationship.

There are many advantages in studying the multiplication of viruses in tumors and free-living tumor cells. One can determine the effect of the virus on tumor growth, and material for cytological examination is readily available. As has already been pointed out, cells of the ascites tumors may be utilized in many ways. Since they can be counted and the virus titered, we may expect more accurate information on some of the fundamental problems of viral multiplication. They also lend themselves to *in vitro* studies. Chemical, cytochemical, and metabolic studies may be used to elucidate some of the puzzling questions which arise in virus and tumor research. Tumor-tissue culture has already taken its place as an established laboratory procedure and is being used in a practical way to titer virus. The direct effect of viruses on tumor cells can be visualized and will undoubtedly lead to a better understanding of both viruses and tumor.

The use of oncolytic viruses for clinical cancer therapy is an intriguing possibility although the chance of therapeutic success seems small. The clinical trials to date have demonstrated that some viruses are sufficiently

innocuous to permit experimental use in man, that in some patients viruses may exhibit selective oncotropism, and that tumor regression will occasionally occur concurrently with virus infection. However, no claim of practical therapeutic usefulness has been made, and it is obvious that the necessity of producing total destruction of all cancer cells during the short period of virus propagation is a massive obstacle to success. The discovery of new and more effective oncolytic viruses, or the adaptation of presently available viruses to greater oncolytic capacity, offers the only hope of clinical success.

LITERATURE CITED

1. Levaditi, C., and Haber, P., *Compt. rend.*, **202,** 2018–20 (1936)
2. Levaditi, C., and Haber, P., *Rev. immunol.*, **3,** 5–24 (1937)
3. Haber, P., and Coquoin, M., *Compt. rend. soc. biol.*, **125,** 238–40 (1937)
4. Levaditi, C., Schoen, R., and Reinié, L., *Compt. rend. soc. biol.*, **124,** 711–13 (1937)
5. Levaditi, C., and Schoen, R., *Compt. rend. soc. biol.*, **125,** 607–9 (1937)
6. Levaditi, C., and Henry-Eveno, J., *Compt. rend.*, **236,** 1383–85 (1953)
7. Bardach, M., *Compt. rend.*, **222,** 1146–48 (1946)
8. Bridré, J., and Bardach, M., *Compt. rend.*, **222,** 1189–91 (1946)
9. Bridré, J., Bardach, M., and Rinjard, P., *Compt. rend.*, **222,** 1415–16 (1946)
10. Rinjard, P., Bardach, M., and Bridré, J., *Compt. rend.*, **222,** 1533–35 (1946)
11. Levaditi, C., and Nicolau, S., *Compt. rend. soc. biol.*, **87,** 498–500 (1922)
12. Levaditi, C., and Schoen, R., *Compt. rend. soc. biol.*, **122,** 736–39 (1936)
13. Moore, A., *Cancer*, **2,** 516–24 (1949)
14. Levaditi, C., and Nicolau, S., *Ann. inst. Pasteur*, **37,** 443–77 (1923)
15. Kritzler, R., Mulliken, B., and Turner, J., *Cancer*, **4,** 401–9 (1951)
16. Koprowski, H., and Norton, T., *Cancer*, **3,** 874–85 (1950)
17. Sharpless, G., Davies, M., and Cox, H., *Proc. Soc. Exptl. Biol. Med.*, **73,** 270–75 (1950)
18. Schoen, R., *Compt. rend. soc. biol.*, **131,** 992–95 (1939)
19. Levaditi, C., and Nicolau, S., *Ann. inst. Pasteur*, **37,** 1–106 (1923)
20. Levaditi, C., and Nicolau, S., *Compt. rend. soc. biol.*, **86,** 928 (1922)
21. Levaditi, C., and Nicolau, S., *Compt. rend.*, **174,** 1649–52 (1922)
22. Levaditi, C., and Nicolau, S., *Ann. inst. Pasteur*, **37,** 443–77 (1923)
23. Levaditi, C., *Rev. immunol.*, **4,** 481–97 (1938)
24. Levaditi, C., and Nicolau, S., *Compt. rend. soc. biol.*, **87,** 2–5 (1922)
25. Levaditi, C., and Schoen, R., *Compt. rend. soc. biol.*, **122,** 736–39 (1936)
26. Rivers, T., and Pearce, L., *J. Exptl. Med.*, **42,** 523–37 (1925)
27. Turner, J., and Mulliken, B., *Cancer Research*, **7,** 774–77 (1947)
28. Turner, J., and Mulliken, B., *Cancer*, **3,** 354–60 (1950)
29. Kritzler, R., Mulliken, B., and Turner, J., *Cancer Research*, **9,** 74–75 (1949)
30. Taylor, A., and Carmichael, N., *Proc. Soc. Exptl. Biol. Med.*, **83,** 676–78 (1953)
31. Schoen, R., *Compt. rend.*, **204,** 1903–4 (1937)
32. Schoen, R., *Compt. rend. soc. biol.*, **128,** 135–38 (1938)
33. Schoen, R., *Ann. inst. Pasteur*, **60,** 499–510 (1938)
34. Schoen, R., *Compt. rend. soc. biol.*, **125,** 845–46 (1937)
35. Schoen, R., *Bull. acad. méd. (Paris)*, **121,** 281–86 (1939)
36. Kuwata, T., *Science*, **114,** 640–41 (1951)
37. Levaditi, C., and Schoen, R., *Compt. rend.*, **202,** 702–4 (1936)

38. Levaditi, C., Schoen, R., and Reinié, L., *Ann. inst. Pasteur*, **58**, 353–76 (1937)
39. Schoen, R., *Compt. rend. soc. biol.*, **125**, 939–41 (1937)
40. Levaditi, C., Schoen, R., and Reinié, L., *Compt. rend. soc. biol.*, **124**, 793–94 (1937)
41. Haguenau, J., Cruveilhier, L., and Viala, C., *Compt. rend. soc. biol.*, **124**, 892–93 (1937)
42. Mellanby, E., *J. Pathol. Bact.*, **46**, 447–60 (1938)
43. Carr, J., *Brit. J. Exptl. Pathol.*, **27**, 1–3 (1946)
44. Moore, A., *Cancer*, **2**, 525–34 (1949)
45. Moore, A., and O'Connor, S., *Cancer*, **3**, 886–90 (1950)
46. Moore, A., *Proc. Am. Assoc. Cancer Research*, **1**, 39 (1953)
47. Moore, A., *Proc. Soc. Exptl. Biol. Med.*, **76**, 749–54 (1951)
48. Moore, A., *Cancer*, **4**, 375–82 (1951)
49. Moore, A., *Ann. N. Y. Acad. Sci.*, **54**, 945–52 (1952)
50. Buckley, S., Buckley, J., and Snipes, A., *Cancer*, **4**, 367–74 (1951)
51. Ginder, D., and Friedewald, W., *Proc. Soc. Exptl. Biol. Med.*, **77**, 272–76 (1951)
52. Ginder, D., and Friedewald, W., *Proc. Soc. Exptl. Biol. Med.*, **79**, 615–18 (1952)
53. Pearce, L., and Rivers, T., *J. Exptl. Med.*, **46**, 81–99 (1927)
54. Pearce, L., and Rivers, T., *J. Exptl. Med.*, **46**, 65–80 (1927)
55. Andrewes, C., *J. Pathol. Bact.*, **50**, 227–34 (1940)
56. Findlay, G., and MacCallum, F., *Trans. Roy. Soc. Trop. Med. Hyg.*, **30**, 507–14 (1937)
57. Southam, C., Bronstein, B., and Webber, L., *Cancer Research*, **11**, 669–75 (1951)
58. MacDowell, E., Taylor, M., and Broadfoot, J., *Carnegie Inst. Wash. Year Book No. 49*, 178–81 (1949–1950)
59. Moore, A., and Stock, C., *Cancer Research*, **10**, 233 (1950)
60. Southam, C., and Epstein, J., *Cancer Research*, **13**, 581–586 (1953)
61. Koprowski, H., Koprowska, I., and Love, R., *Proc. Nat. Acad. Sci.*, **39**, 1147–48 (1953)
62. Kausche, G., Landschutz, C., and Sauthoff, R., *Z. Naturforsch.*, **7b**, 33–36 (1952)
63. Moore, A., and Diamond, L., *Proc. Soc. Exptl. Biol. Med.*, **79**, 663–69 (1952)
64. Moore, A., and Diamond, L., *J. Immunol.*, **71**, 441–45 (1953)
65. Moore, A., Diamond, L., Mackay, H., and Sabachewsky, L., *Proc. Soc. Exptl. Biol. Med.*, **81**, 498–501 (1952)
66. Price, A., and Ginsberg, H., *Federation Proc.*, **12**, 455–56 (1953)
67. Koprowska, I., and Koprowski, H., *Cancer Research*, **13**, 651–60 (1953)
68. Love, R., Koprowski, H., and Cox, H., *Cancer Research*, **13**, 350–57 (1953)
69. Ackermann, W., and Kurtz, H., *Proc. Soc. Exptl. Biol. Med.*, **81**, 421–23 (1952)
70. Koprowska, I., and Koprowski, H., *Federation Proc.*, **11**, 420 (1952)
71. Koprowska, I., and Koprowski, H., *J. Natl. Cancer Inst.*, **14**, 627–43 (1953)
72. Cheever, F., and Dickos, J., *Proc. Soc. Exptl. Biol. Med.*, **83**, 822–25 (1953)
73. Findlay, G., and MacCallum, F., *Brit. J. Exptl. Pathol.*, **21**, 173–80 (1940)
74. Hallauer, C., *Z. Hyg. Infectionskrankh.*, **113**, 61–74 (1931)
75. Harrison, R., and Julianelle, L., *Am. J. Ophthalmol.*, **20**, 360–67 (1937)
76. Bang, F., and Gey, G., *Trans. N. Y. Acad. Sci.*, **13**, 324–27 (1951)
77. Gey, G., and Bang, F., *Trans. N. Y. Acad. Sci.*, **14**, 15–24 (1951)
78. Bang, F., and Gey, G., *Bull. Johns Hopkins Hosp.*, **91**, 427–62 (1952)
79. Scherer, W., *Am. J. Pathol.*, **29**, 113–37 (1953)
80. DeLustig, E., and Parodi, A., *Rev. soc. argentina biol.*, **27**, 324–30 (1951)
81. DeLustig, E., and Parodi, A., *Compt. rend. soc. biol.*, **146**, 612 (1952)

82. Scherer, W., Syverton, J., and Gey, G., *J. Exptl. Med.*, **97**, 695–710 (1953)
83. Stoler, M., and Gey, M., *Bull. Johns Hopkins Hosp.*, **92**, 385–87 (1953)
84. Scherer, W., *Proc. Soc. Exptl. Biol. Med.*, **80**, 598–602 (1952)
85. Pollard, M., and Russell, R. H., *Proc. Soc. Exptl. Biol. Med.*, **80**, 574–78 (1952)
86. Toolan, H., and Moore, A., *Proc. Soc. Exptl. Biol. Med.*, **79**, 697–702 (1952)
87. DePace, N., *La Ginecologia*, **9**, 82–88 (1912)
88. Salmon, P., and Baix, *Compt. rend. soc. biol.*, **86**, 819–20 (1922)
89. Southam, C., and Moore, A., *Am. J. Trop. Med. Hyg.*, **31**, 724–41 (1951)
90. Erf, L., *The Penna. Med. J.*, **53**, 1172–81 (1950)
91. Bierman, H., Crile, D., Dod, K., Kelly, K., Petrakis, N., White, L., and Shimkin, M., *Cancer*, **6**, 591–605 (1953)
92. Hoster, H., Zanes, R., and von Haam, E., *Cancer Research*, **9**, 473–80 (1949)
93. Southam, C., and Moore, A., *Cancer*, **5**, 1025–34 (1952)
94. Higgins, G., and Pack, G., *Bull. Hosp. Joint Diseases*, **12**, 379–82 (1951)
95. Pack, G., *Arch. Dermatol. u Syphilol.*, **62**, 694–95 (1950)
96. Blank, H., *Acta Dermato-Venereol.*, **29**, 77–107 (1949)
97. Southam, C., and Moore, A., *Am. J. Trop. Med. Hyg.*, **3**, 19–50 (1954)
98. Koprowska, I., *Am. J. Pathol.*, **29**, 1105–21 (1953)

BACTERIAL TOXINS[1]

Demonstration of Antigenic Components in Bacterial Filtrates

By C. L. Oakley

Department of Bacteriology, The University, Leeds, England

In 1950 van Heyningen published in his book, *Bacterial Toxins*, a thorough and able review of the subject up to 1949 (1); however much I may disagree with him on trifles, with his treatment of the subject, and with his general conclusions, I am in almost complete agreement. It seems hardly useful, therefore, to reprint here the substance of his review, even in a considerably compressed form, but rather better to deal with some aspects of the matter which van Heyningen, in addressing his book to biochemists, had no need to discuss, and with some others in which recent experimental work has extended our knowledge or thrown doubt on older views.

The Word "Toxin"

In the early days of work on bacterial products the word "toxin" had a fairly precise meaning, a poisonous substance of high molecular weight produced by bacteria; to this was later added the property of antigenicity, and toxins were divided into two groups: exotoxins, secreted by the organisms into the medium in which growth was occurring, and endotoxins which formed part of the body of the organism, from which they could be separated only by autolysis or other destructive procedures. As the concept of toxins was extended from that of lethal substances to those bacterial products that could cause dermonecrosis, haemolysis, breakdown of collagen, and so on, two types of opinion became common: one in which all the toxic activities of a bacterial filtrate were ascribed to a single substance, a view enshrined in the application of the word "toxin" to a toxic bacterial filtrate, and another in which each toxic activity was supposed to be attributable to a different substance. Neither of these views is entirely true, for it has been shown that a toxic bacterial filtrate may contain substances with one known property or with several, while several substances with the same activity may be present. Thus filtrates of *Clostridium welchii* Type C may contain five substances that are lethal under suitable conditions [Glenny *et al.* (2); Oakley *et al.* (3)]; one of these, alpha toxin is a necrotising calcium-dependent lecithinase, haemolytic to the red cells of most laboratory animals except horses and goats; beta toxin is necrotising but not haemolytic: gamma toxin is lethal only; delta toxin is haemolytic only to the red cells of even-toed ungulates and is not dependent on calcium for haemolysis; kappa toxin is a collagenase and gelatinase. The oxygen-labile lysin (theta toxin) may also be lethal in high concentrations.

[1] The survey of the literature pertaining to this review was completed in December, 1953.

Beside this, the distinction between exotoxins and endotoxins is far less clear than it once was, for it has been shown that substances having the properties of exotoxins (heat lability, high antigenicity, easy toxoiding with formalin) can be obtained in high concentration by allowing certain bacteria to autolyse, or by extracting bacterial bodies with various solvents [botulinum toxin, Nelson (4) and Raynaud & Second (5); tetanus toxin and *C. sordellii* toxin, Raynaud (6)].

Moreover, as van Heyningen has pointed out, the toxicity of a bacterial antigen may appear to be very low because it is present in very small amounts in the preparation used; it may quite possibly be true that such substances as *C. welchii* and *C. septicum* desoxyribonuclease are as toxic weight for weight as the well-known lethal toxins of these organisms; purification studies are not sufficiently advanced to settle this question. However van Heyningen & Gladstone (7) have shown, for example, that the neurotoxin of *Shigella dysenteriae*, which would not ordinarily be regarded as highly lethal, is weight for weight as toxic as the toxin of *C. botulinum* Type A.

It seems to me, therefore, that the word "toxin" has largely outlived its usefulness and that it might profitably be replaced by "soluble bacterial antigen," though it is so thoroughly embedded in the literature and has so fine a sound, that it is very unlikely, for all its confusing associations, to be displaced. The complex protein-polysaccharide-lipoid complexes that are much less readily extractable from bacteria might well be called "Boivin antigens"; they will not be further discussed in this review.

DISCRIMINATING METHODS FOR SOLUBLE BACTERIAL ANTIGENS

The average bacterial filtrate is exceedingly complex; it contains not only substances derived from the bacterium, but substances, sometimes antigenic, derived from the medium in which it is growing. Luckily the latter substances seldom have any recognisable activity other than the capacity to flocculate with antisera prepared against the filtrate, so that we can usually confine ourselves to antigens of bacterial origin. Van Heyningen did not give any account of the methods by which the presence of soluble antigens is demonstrated in complex mixtures, and this review seems a convenient place to systematise the scattered literature on the subject.

Dilution Methods

Bacterial filtrates have so far been shown to possess either no indicating effect at all, or one or more of the following activities: lethality, dermonecrosis, flush production on intradermal injection, neurotoxicity of various kinds, haemolytic, haemagglutinating, lecithinase, collagenase, gelatinase, lipase, hyaluronidase, proteinase, or desoxyribonuclease activity. Clearly if in any one filtrate, only one substance were responsible for all the activities observed, the dilutions of a filtrate necessary to produce a specified indicating

effect would always run parallel, whatever filtrate was used. Thus if a filtrate possessing lethal and haemolytic properties required dilution to 1 in 10 to produce a standard lethal effect, and to 1 in 250 for a standard haemolytic effect, then another filtrate (from the same organism) whose effective dilution in a lethal test was 1 in 50 should require dilution to 1 in 1250 in a haemolytic test. Unfortunately activity against different indicators does not always run parallel in toxic filtrates whose activities are almost certainly attributable to the same antigen; thus some filtrates of cultures of *C. tetani* are 300 times as toxic (on an equal weight basis) to guinea pigs as to rabbits: other preparations are only three times as toxic [Llewellyn-Smith (8)]. Moreover, if more than one substance effective in the test are present, the results obtained in dilution tests will depend largely on their relative concentrations. Suppose for instance that one haemolytic and lethal substance is present, with another that is haemolytic only. If the latter is present in small amounts in all filtrates, the haemolytic end-point in dilution tests will always be determined for the haemolytic and lethal substance, and the haemolytic and lethal effects may be ascribed to the same substance. If, however, the purely haemolytic substance is present in high concentration, it will govern the determination of the haemolytic end-point, and haemolysin and lethal substance will be supposed to be distinct. Both conclusions are incomplete, and only if filtrates of varying composition are available is the truth likely to be suspected.

Activity determinations are particularly likely to mislead during purification studies; if two haemolysins are present in a mixture, effective at dilutions of 1 in 10 and 1 in 100 respectively, and the purification process yields 100 per cent of the first and none of the second haemolysin, dilution tests may suggest that the total loss of haemolysin is 90 per cent and the increase in purification of the first haemolysin may pass unnoticed.

In some instances components in mixtures may be isolated by physical or chemical means and their activites examined separately in dilution tests. Thus oxygen-labile lysins in a mixture may be inactivated by shaking with oxygen or by ageing in contact with air [Herbert & Todd (9)], by adsorption on cholesterol (or on almost any 3-hydroxy-sterol of β-configuration) [Howard *et al.* (10)], or by treatment with certain normal sera. Haemolysins that are not oxygen-labile may be estimated separately. *C. welchii* epsilon and iota toxins are activated by trypsin [Bosworth & Glover (11, 12); Ross *et al.* (13)] a process that destroys some of the toxins associated with them. Some proteinases [e.g., *C. welchii* lambda toxin, Oakley *et al.* (3)] may be inactivated with fresh normal sera or with citrate and the unaffected proteinases dealt with separately; activation of some proteinases with cysteine may notably alter the properties of the mixture in which they occur [Oakley & Warrack (14)].

Discrimination by dilution techniques may occasionally be advanced by the use of more specific indicators. Haemolysins, for example, are often strikingly limited in the species of red cell they will attack. Thus *C. welchii*

alpha toxin attacks sheep red cells, but not those of horses or goats (it seems strange that sheep and goats, so similar zoologically, should differ so much in their red cells); *C. welchii* theta toxin and the other oxygen-labile lysins are relatively ineffective against mouse cells [Oakley (15); Howard & Wallace (16)], though they readily haemolyse most others; of available laboratory animals, *C. welchii* delta toxin affects the red cells of only cows, pigs, goats, and sheep.

Even with these refinements, however, dilution methods lack specificity, and the only methods in which specificity has so far been taken account of are immunological ones.

Immunological Discrimination

Indicator methods.—If a mixture of antigenic substances, either crude or treated in some way, e.g., by toxoiding with formalin, that will reduce its toxicity without altering its antigenicity, is injected into a mammal or bird, the injected animal, will, with suitable treatment produce a complex mixture of antibodies, at least one for each antigen. The ratios between the concentrations of the various antibodies will, however, not necessarily be constant in different animals injected with the same filtrate and may be very different indeed in animals injected with filtrates obtained from the same organism grown in different media or under different conditions. The reason for the variable antibody response to filtrates obtained under different conditions is clear enough: the filtrates may vary greatly in composition. Thus if *C. welchii* Type A is grown in a simple meat-broth medium plus 1 per cent peptone and 0.5 per cent glucose, theta toxin is produced early in large amounts; alpha toxin is produced later. In a similar medium with added meat particles, alpha toxin is produced in far larger amounts, but no theta toxin at all [cf. Schnayerson & Samuels (17)]; the concentration of kappa toxin (collagenase) is unaffected. Injection of such different filtrates into comparable horses is likely to evoke the production of antibodies to these three antigens in very different ratios.

The factors that determine the relative production of antibodies in horses injected with the same filtrate are much less well understood, but one of them is certainly the possession by the injected animal of "natural" antibody. Thus many horses possess "natural" diphtheria antitoxin [Glenny (18)] or natural *C. welchii* alpha or theta antitoxin as a result in most cases, no doubt, of previous infection with *Corynebacterium diphtheriae*, *C. welchii*, or related organisms. Such animals respond much more readily to the antigens to which they already have natural antibody and will, when injected with a mixture of antigens, yield sera containing proportionately far more antibody against those antigens of which they have had previous experience. Unfortunately the antibodies so produced against the other antigens may be nonavid and consequently of little value for discrimination [Glenny *et al.* (19, 20, 21); Jerne (22)].

To produce "avid" antibodies against antigens to which the animals

have no natural immunity requires preliminary immunisation (with alum-precipitated antigens or toxoids, or the antigens or toxoids alone) followed by a period of rest of varying length before definite immunisation is resumed. In general the longer the resting period the stronger and more avid the antibodies produced [Glenny *et al.* (23); Barr & Glenny (24)]. Even in rested animals the ratios between antibodies may vary greatly, especially at different times during immunisation; thus in the early stages of immunisation against *C. welchii* Type C, the ratios between alpha or delta antitoxins and beta antitoxin may be very different from their values in the later stages [Glenny *et al.* (2)].

Where there is evidence that related bacteria produce slightly different sets of antigens, these may be used to produce antisera of varying composition. Thus *C. welchii* Type A filtrates will evoke in suitably rested animals antibodies to alpha, theta, kappa, mu, and nu antigens; of these five antibodies four may be used to neutralise four antigens (alpha, theta, kappa, nu) of *C. welchii* Type C, leaving beta, gamma, delta available for test. Of these beta and gamma may be neutralised with *C. welchii* Type B antisera, leaving delta alone effective in tests. Sera in which the ratio of one antibody to the others in which one is interested is very high are called monovalent or monospecific antisera [or more regrettably, "pure" antisera, Oakley (15)] and are very valuable for discriminatory purposes.

Such monospecific sera can occasionally be used to remove antigens, either by neutralisation or by flocculation, from filtrates used to immunise animals. Thus *C. welchii* Type D antisera (containing mainly alpha and epsilon antitoxins) could be used to remove alpha and epsilon antigens from *C. welchii* Type B filtrates, which would then evoke in suitable animals antisera less potent against alpha and epsilon toxins (and possibly other antigens for which at present we have no indicating systems) than the untreated filtrates would. All these methods, however, require some knowledge of the antigenic composition of filtrates and the testing of antisera; we may now show how this is obtained.

Testing of antisera.—The basis of all modern testing of avid antisera for their values against antigens is a comparison of the volume of serum (as compared with that of a standard serum) that will under constant conditions reduce the activity of a quantity of a toxic filtrate to a certain fixed level, the standard indicating effect. Thus for a lethal antigen, this effect might be taken as that of the LD_{50}, i.e., death of half the animals injected within, say, 48 hr. The standard and the unknown serum would then be compared by determining the volume of each which must be added to several LD_{50} of the filtrate to reduce its activity to that of 1 LD_{50}. It is evident that the quantity of filtrate used must considerably exceed the LD_{50} if any great accuracy is to be obtained; 30 to 50 LD_{50} in the test-dose gives satisfactory results, though fewer may be used if the values of the sera are very low.

For other activities other standard indicating effects may be chosen: for

necrotising filtrates, the production of a necrotic lesion of a particular size, for filtrates evoking a flush on intradermal injection, a flush of a particular size; for haemolytic filtrates, haemolysis of x per cent of a y per cent suspension of susceptible red cells; for filtrates with enzymatic activities, the breakdown of a certain percentage of substrate determined either directly or indirectly. In all cases the 30 to 50 indicating doses in the test dose will determine the level of test, i.e., the number of units of antitoxin in the "neutral" mixture, for this will clearly be the amount of the antitoxin that will neutralise all but one indicating dose of the filtrate.

The standard serum used may be an international standard, where one exists; if no such standard is available a laboratory standard possessing high neutralising ability should be chosen, and given an arbitrary value of so many "units" per ml. If its value against some antigen already described is known, it is often convenient to ascribe this value to the standard in tests against unknown antigens; comparison of values is usually facilitated. It is essential, for comparison between standard and test serum to be valid, that the curves of residual antigen plotted against log volume of antiserum added should be parallel for the sera compared and that the parallelism should be unaffected by dilution of the mixtures.

Serological Discrimination

The use of numerous indicators.—It is evident that if a filtrate contains a single antigenic substance with several indicating effects, the values of sera against that filtrate will be independent of the indicator used. Significant discrepancies between serum values against the same filtrate using different indicators should always suggest the presence of more than one antigen.

Suppose, for example, that a filtrate A contains two antigens, one (alpha) haemolytic, necrotising, and lethal, and another (beta) necrotising and lethal but not haemolytic, and that the standard has by luck been so chosen that the test dose of filtrate contains an amount of each component just neutralised by the same volume of standard. Tests at 1 unit may then be regarded as giving either the alpha or beta end point. The "real" values of the sera are those that would be obtained if they were assayed against a single component of the mixture.

As Table I shows, sera W and X have equal values in tests with all three indicators; one component of the filtrate is probably haemolytic, necrotising, and lethal. Two sera (V, Y) though they give equal values in necrotising and lethal tests, give higher values in haemolytic tests; another lethal and necrotising antigen is probably present, but it is not haemolytic; if it were the same values would be obtained in haemolytic, necrotising, and lethal tests (i.e., the values of sera V, Y in haemolytic tests would be 150 and 0). Serum Y has no antibody to beta, but has some to alpha; Z certainly has none to alpha, and may have none to beta; both of these sera will be of value in later tests.

The position in these tests is somewhat artificial, as in practice the end-

TABLE I*

VALUES OF SERA IN TESTS AGAINST FILTRATE A USING DIFFERENT INDICATORS†

Sera	Real Values		Estimated Values Using as Indicator		
	Anti-α	Anti-β	Haemolysis	Necrosis	Death
Standard	100	100	100	100	100
V	3000	150	3000	150	150
W	50	300	50	50	50
X	400	1000	400	400	400
Y	500	0	500	0	0
Z	0	250	0	0	0

* In this and in the remaining tables, statistical variation in the determination of serum values is for convenience ignored.

† The test dose is equivalent to 1α and 1β unit.

points for two components in tests against the standard seldom coincide. Table II gives the results to be expected if the test dose at 1 unit contains the equivalent of 1 unit alpha and 0.1 unit beta antitoxin (filtrate B).

Conclusions similar to those derived from Table I may be drawn from Table II.

TABLE II

VALUES OF SERA AGAINST FILTRATE B USING DIFFERENT INDICATORS*

Sera	Real Values		Estimated Values Using as Indicator		
	Anti-α	Anti-β	Haemolysis	Necrosis	Death
Standard	100	100	100	100	100
V	3000	150	3000	1500	1500
W	50	300	50	50	50
X	400	1000	400	400	400
Y	500	0	500	0	0
Z	0	250	0	0	0

* The test dose is equivalent to 1α and 0.1β unit.

A somewhat more interesting situation arises when the filtrate contains such a proportion of antigens that the test dose contains 10 equivalents of beta to one of alpha; the test dose in haemolytic tests will then be equivalent to 1 alpha and 10 beta units; that in necrotising or lethal tests to 0.1 alpha and 1 beta unit (filtrate C).

All that can be determined from Table III is that there is a lethal and necrotising antigen present distinct from the haemolysin; that the haemo-

TABLE III

VALUES OF SERA AGAINST FILTRATE C USING DIFFERENT INDICATORS*

Sera	Real Values		Estimated Values Using as Indicator		
	Anti-α	Anti-β	Haemolysis	Necrosis	Death
Standard	100	100	100	100	100
V	3000	150	3000	150	150
W	50	300	50	300	300
X	400	1000	400	1000	1000
Y	500	0	500	0	0
Z	0	250	0	0	0

* The test dose is equivalent to 1α and 10β units in haemolytic tests, and to 0.1α and 1β unit in necrotising or lethal tests.

lytic antigen is also necrotising and lethal cannot be deduced.

The use of numerous filtrates.—Comparison of the results in Tables I, II, III also shows the effect of assaying sera against filtrates of different composition. Another example may be given; by growing *C. welchii* Type A in meat broth with added meat particles we may obtain a filtrate whose haemolytic activity is entirely attributable to alpha toxin; if we grow it in meat broth without added meat, a mixture of alpha and theta haemolysins is obtained. Haemolytic tests would then give the kind of results shown in Table IV.

Tests against blocked filtrates.—If the filtrates used in the tests on the right of Table IV were allowed to age in contact with air, or were shaken

TABLE IV

VALUES OF SERA IN HAEMOLYTIC TESTS AGAINST TWO C. WELCHII TYPE A FILTRATES*

Sera	Real Values		Estimated Values Against Filtrate with Test Dose Equivalent to	
	Anti-α	Anti-θ	1α, 0θ Unit	0.5α, 1θ Unit
Standard	100	100	100	100
G	1000	10	1000	10
H	50	100	50	100
J	70	1500	70	140
K	30	400	30	60
L	0	30	0	0
M	150	0	150	0

* Note that when the end-point is determined for the same component in filtrates of different composition, the serum values are in constant proportion (cf. results for sera H, J, K).

with oxygen, the theta toxin would be inactivated, and the tests against both filtrates would give equal results for the sera. Alternatively, alpha toxin might be blocked with phosphate or oxalate, without affecting theta; the left-hand filtrate would then show no haemolytic activity; the values for sera G, H, J, K, M, against the right hand filtrate would be unaltered while that of L would be increased to 30.

Occasionally the indicator can be so chosen that the activity of one or more components is blocked. Thus the results in Table IV would be obtained only if sheep red cells were used (of the group sheep, horse, goat); if horse or goat red cells were used, only theta toxin would be active, and the antitheta values of the sera would be obtained. Similarly if sera were tested against a mixture of *C. welchii* alpha, theta, and delta toxins, using sheep cells as indicator, the results would depend on the relative proportions of the toxins and antitoxins, as all three toxins affect the indicator; but if horse cells were used, theta toxin only would be effective, and the antitheta values of the sera could be determined; if goat cells were used, theta and delta would be effective; if rabbit cells, alpha and theta only, with corresponding effects on the values of the sera tested.

Much more useful testing can, however, be done with filtrates in which one or more components are blocked with monospecific antisera. It is evident, for instance, that if two antigenic components of a filtrate are effective in a test, addition to the filtrate of excess of an antiserum that possesses antibody to one component only will limit the activity of the filtrate in the test to the other component, against which other sera may be tested. If m components are effective in the test, and the serum possesses antibodies to n of these, it will reduce the activity of the filtrate to m-n components, some of which may be neutralisable by other sera to further simplify testing.

Thus if the filtrate referred to in Table I is overneutralised with more than 0.002 ml. of serum Y per test dose, all the haemolytic activity of the filtrate will be lost, though it will retain its necrotising and lethal activity; tests of sera against these activities will show that they are a result of the same component (beta). A more complex example is given in Table V, where three components are present in the filtrate D; one (alpha) is haemolytic, produces opalescence in filtered egg yolk emulsions [Macfarlane *et al.* (25)], and is lethal; beta is opalescing only; gamma is haemolytic only. For convenience the test dose is supposed to be equivalent to 1 alpha, 1 beta, or 1 gamma unit and the blocking serum to contain alpha antitoxin only.

There is sufficient evidence in the Table that an opalescing substance and a haemolytic substance distinct from alpha are present and that they are distinct from one another. Since the filtrate loses its lethal but not its opalescing and haemolytic power when alpha antitoxin is added, it is very tempting to suggest that alpha toxin is lethal only, but as addition of alpha antitoxin markedly alters the values of sera against the filtrate in haemolytic and opalescing tests, it is probable that alpha is haemolytic and opalescing as well as lethal.

TABLE V

VALUES OF SERA AGAINST COMPLEX FILTRATE D, WITH AND WITHOUT BLOCKING WITH AN ANTI-α SERUM CONTAINING NO ANTI-β OR ANTI-γ*

Sera	Real Values			Estimated Values in					
				Haemolytic Tests		Opalescing Tests		Lethal Tests	
	Anti-α	Anti-β	Anti-γ	Unaltered	-α†	Unaltered	-α	Unaltered	-α
Standard	250	250	250	250	250	250	250	250	—‡
H	1000	500	200	200	200	500	500	1000	—
J	100	300	700	100	700	100	300	100	—
K	600	100	250	250	250	100	100	600	—
L	500	300	400	400	400	300	300	500	—
M	800	600	250	250	250	600	600	800	—
N	250	150	50	50	150	150	150	250	—
P	200	1000	100	100	100	200	1000	200	—

* Test dose equivalent to 1α, 1β, 1γ unit.
† Neutralized with anti-α serum.
‡ Filtrate not lethal.

TABLE VI

SERUM VALUES AGAINST A COMPLEX SYSTEM E, WITH AND WITHOUT BLOCKING WITH AN ANTI-α SERUM*

	Real Values			Estimated Values in					
				Haemolytic Tests		Opalescing Tests		Lethal Tests	
	Anti-α	Anti-β	Anti-γ	Unaltered	-α†	Unaltered	-α	Unaltered	-α
Standard	250	250	250	250	250	250	250	250	—‡
H	1000	500	200	400	200	1000	500	1000	—
J	100	300	700	100	700	100	300	100	—
K	600	100	250	500	250	500	100	600	—
L	500	300	400	500	400	500	300	500	—
M	800	600	250	500	250	800	600	800	—
N	250	150	50	100	50	250	150	250	—
P	200	1000	100	200	200	200	1000	200	—

* Tests doses as in text.
† Neutralised with anti-α serum.
‡ Filtrate not lethal.

If the test dose at 1 unit were equivalent to 1 alpha, 0.2 beta, and 0.5 gamma unit, the results in Table VI would be obtained; when alpha toxin is neutralised, the redetermined test dose will be equivalent to 1 beta and 2.5 gamma units in opalescing tests and to 1 gamma and 0.4 beta unit in haemolytic tests.

It is evident that in the tests in Table V, overneutralisation of the filtrate will not alter the test doses against the standard significantly, except in lethal tests, in which the blocked filtrate has no lethal activity. In the tests in Table VI, on the other hand, the test dose in haemolytic tests will be doubled and that in opalescing tests multiplied by five. Consequently in any test in which the same toxin is effective at the end point in both blocked and unblocked filtrate, the value of the serum will be reduced to half in haemolytic, or to one-fifth in opalescing tests (e.g., sera H, K, M, N, in haemolytic tests, and sera K, P, in opalescing tests in Table VI). Conversely if the value of a serum against a blocked filtrate shows a change, as compared with its value against the unblocked filtrate, in inverse proportion to the change in the test dose, the component for which the end point is determined is most likely to be the same in both cases; and sera like J, L, P, which do not show this change in haemolytic tests, are probably neutralising different components at the end-point in blocked and unblocked filtrates (cf. also all sera except K, P, in opalescing tests).

Similarly if a filtrate is treated with a substance that activates one component without affecting the others the test dose may be reduced and the values of some sera may be correspondingly increased. If one component is activated and others inactivated considerable ingenuity may be necessary in interpreting the results, but the general argument will be very similar to that used above.

For convenience I have grouped most of the available methods together and systematised them. Examples may be found in the following papers: general [Oakley (15)]; *Clostridium histolyticum* [Oakley & Warrack (14), Evans & Prophet (26)]; *C. oedematiens* [Oakley *et al.* (27)]; *C. septicum* [Bernheimer (28), Warrack *et al.* (29)]; *C. welchii*: general [Oakley 15)]; Type A [Dalling *et al.* (30), Glenny *et al.* (2, 31), Glotowa *et al.* (32), Hottle & Okono (33), Macfarlane *et al.* (25), Oakley *et al.* (3, 34, 35)]; Types A, B, C, D [Glenny *et al.* (2, 31), Oakley *et al.* (3), Oakley & Warrack (36)]; Type E [Bosworth (37)]; Type F [Oakley (38)]; *Staphylococcus aureus* [Burnet (39), Glenny & Stevens (40), Llewellyn-Smith & Price (41, 42)].

Flocculation methods.—All the methods so far dealt with require an external indicator for toxin or antigen excess and are, therefore, inapplicable to antigens for which no indicator has yet been devised; these are no doubt the great majority. For some of these the flocculation method is available.

If a soluble bacterial antigen possessing some indicating activity and the corresponding horse antibody are mixed in appropriate proportions and concentrations, the mixtures first become opalescent and later develop a floccular precipitate; in the ideal case the mixture that flocculates first is the one most nearly neutral in indicating tests. If time of flocculation is plotted

as ordinate against quantity of antiserum added to constant amount of filtrate as abscissa, the graph obtained is a parabola-like curve with its vertex downwards and with little lateral spread [Ramon (43); Glenny & Wallace (44); Glenny & Pope (45)]. If the test materials contain a mixture of antigens and antibodies respectively, then for each system present in appropriate concentration there will be a parabolic curve with an inverted vertex at the neutral point or flocculation optimum: the neutral point for antigens possessing no indicating capacity being that (it is assumed) at which no free antigen or antibody is present in the mixture. Obviously even if there were no overlapping of the curves it would not be possible to ascribe any particular vertex to a particular antigen without further investigation, though sometimes the appearance of the floccules may give a clue to the system in which one is interested. If, however, the values of several sera against a particular antigen known to be present are known, and if these sera are diluted to constant concentration of this antibody, the vertices for this antigen-antibody system should coincide in mixtures of the filtrate and any of the sera. Blend flocculation [Glenny & Okell (46)] may greatly assist in discrimination.

Moreover if the optimal flocculation vertices were well separated, i.e., they contained filtrate and antiserum in very different relative concentrations, it might be possible to flocculate out one component from a mixture without gross admixture of the others. The floccules so obtained could be washed with ice cold saline and digested with pepsin at pH 3 to yield some of their antibodies. The supernatant fluids from the floccules can be examined for residual antigens, and any antibodies recovered from the floccules can be used for blocking in indicator tests.

There has been a general tendency to regard the results of flocculation tests as of more fundamental importance than those of indicator tests [*cf.* Cohn (47)], no doubt because, beside being simpler to carry out, they do not suffer from the effects of nonavidity and allow estimation of floccule nitrogen content by chemical means. On the latter method, for example, Pappenheimer & Robinson (48) have based methods for determination of nitrogen content of diphtheria toxin and antitoxin. It has long been known, however, that some sera will not flocculate at the point expected from their inhibiting effect on the indicating activities of antigens, and that some, though they possess considerable antitoxic activity, will not flocculate at all with their antigen [Glenny *et al.* (23); Glenny & Wallace (49); Glenny (50); Barr & Glenny (51)]. It has always seemed to me that conclusions drawn from floccule composition were open to serious objections: they assume that the floccules are uncontaminated with other flocculating systems, that flocculation is complete, and that no extraneous sources of nitrogen are adsorbed to the floccules [see van Heyningen & Bidwell (52); Pope *et al.* (53); Pope & Stevens (54) for discussion of these methods]. It has also been assumed that antibody obtained from floccules is pure; certainly it is usually purer than the antiserum from which it is derived, but judging from the work of Pope

et al. (53), diphtheria antitoxin recovered from diphtheria toxin-antitoxin floccules is still contaminated with other antibodies.

Diffusion methods.—Considerable advances have been made possible by the work of Ouchterlony, Elek and Oudin in adapting the flocculation reaction to antigenic analysis by diffusion [see Oakley & Fulthorpe (55) for earlier work]. Ouchterlony (56 to 59) showed that if an organism was grown on a solid medium as a linear streak at right angles to a rectangular trench of antiserum-agar, flocculation occurred between antigens diffusing from the colony and antibodies diffusing from the antiserum, provided both were present in sufficient concentration. The angles to the colony at which the flocculation lines developed depended on the relative concentration of antigens and antibodies, and on their diffusion coefficients. Moreover if two strains of organisms were grown in parallel across the trench, and they produced souble antigens in common, the corresponding flocculation lines fused betweeen the colonies, while those attributable to unshared antigens passed undeviated across the plate.

As the lines appeared to behave independently, it was natural to assume that each line was a result of a single antigen-antibody system, though overlapping might reduce the number indicated. The method was largely used by Ouchterlony and by Elek (60, 61) for estimating the toxigenicity of *C. diphtheriae* cultures, and by Elek & Levy (62) for examination of toxigenic staphylococci. Obviously no line can be ascribed to a particular antigen without further work, but Elek (61) has shown that information about the activities of components can be obtained fairly easily and used for discrimination. If an indicating antigen flocculates with its antibody along a line, then free antigen should be present on the side of the line away from the antibody source, and free antibody on the other; if the indicator is incorporated in the medium, it will be affected only as far as the flocculation line corresponding to the antigen. If several antigens affecting the same indicator are present (e.g., several haemolysins produced by colonies growing in a medium containing susceptible red cells) the effect on the indicator may be graded as the flocculation lines are crossed. The methods have also been applied to filtrates by Ouchterlony, by allowing filtrate and serum to diffuse towards one another from pits cut in agar plates Comparisons between filtrates and between sera are thus possible, and the filtrates can be modified by blocking some of their components with specific antisera [Bjorklund (63)], but though some quantitative work was done [Elek (60); Ouchterlony (64)], it was usually very rough.

Oudin (65 to 69) had already developed diffusion of relatively pure antigens and rabbit antibodies against one another in columns and elaborated methods for the identification of the antigens responsible for the precipitation zones produced when Oakley & Fulthorpe (55) worked out a quantitative method for examining diffusion systems in columns. In their method, the horse antiserum gelled with agar formed the bottom of the column, the central part was a layer of agar, and the filtrate was poured on top. If anti-

gen and antibody were both present in the system in suitable concentration, a flocculation disc (or "line") appeared in the central column. If the filtrate was diluted, the corresponding line appeared higher up and fainter in the central part of the column. Since addition of antisera to the filtrate in the top would reduce the concentration of free antigen, sera could be assayed against a standard antiserum for their capacity to "move" a particular line upwards through the central column, and more particularly through its upper meniscus, when they were added to the filtrate before it was placed on the top of the column. Discs appearing in systems involving bacterial filtrates and their antisera were thus shown to be attributable in many cases to well-known bacterial toxins and their antibodies, and in some instances where more than one line was produced, each could be ascribed to a particular antigen; no evidence was obtained that any line was a result of Liesegang ring formation. Systems producing more than three lines could seldom be attacked directly, though techniques applied in ordinary discriminatory analysis could still be used, e.g., blocking with monospecific sera, the use of different lines as different indicators, and the use of various sera in the bottom of the column. Evidently if the serum used in the bottom contains only n antibodies, and there are in the filtrate beside the corresponding n antigens, m others, the maximum number of discs produced will be n; there may be fewer because of reduction by overlapping or by failure of antigen or antibody to reach the necessary concentration.

It will be evident that where no ordinary indicating effect is available for checking the identity of an antigen, diffusion methods allow it to be defined in two ways only: by its diffusion rate in Oudin-type columns, and in terms of the values of sera tested against it in the top of columns including a central agar cylinder, using flocculation as the external indicating effect.

"PURE" TOXINS

Everyone uses the word "pure" with his own reservations, and the word is in consequence becoming of doubtful value. There is no question that many bacterial toxins have been purified to a considerable extent, and that some, tetanus toxin [Pillemer *et al.* (70); Pillemer (71)] and *C. botulinum* Type A toxin [Lamanna *et al.* (72, 73); Abrams *et al.* (74)], have been crystallised; but there is good evidence that no preparation so far obtained is immunologically pure, i.e., free from other antigenic material. Indeed Lowenthal & Lamanna (75) have already shown that, since the haemagglutinating and neurotoxic activities of *C. botulinum* Type A filtrates are separable, crystalline *C. botulinum* Type A toxin, which possesses both activities must be impure. Most workers had, however, supposed that the amount of impurity in the best diphtheria toxin preparations was very small [less than 5 per cent Pappenheimer (76); Cohn & Pappenheimer (77)], until Pope *et al.* (53) suggested that substantial amounts of antigenic impurity might be present.

These diphtheria toxin preparations had been regarded as nearly pure because they were of reasonably constant composition, were homogeneous in

the ultracentrifuge, and almost so in the electrophoresis apparatus, because they contained very little bacterial protein, and because they contained about 2170 Lf. per mg. nitrogen [Pappenheimer (76); Pappenheimer *et al.* (78)], a figure to be expected from the contribution of toxin to toxin-antitoxin floccules [Pappeheimer & Robinson (48)]. Pope *et al.* (53) first showed that when crude diphtheria toxic filtrates are allowed to diffuse against crude diphtheria antitoxic serum in colums with a central agar cylinder, as many as 24 lines (ascribed by them to at least as many antigen-antibody reactions) developed; toxin preparations of purity equal to that of Pappenheimer's (76) and one of Pappenheimer's own preparations gave up to 14 lines. These purified preparations were therefore presumably contaminated with at most 13 other antigens, whose total concentration could not, of course, be determined by this method.

Pope *et al.* (53) were unable, moreover, to confirm the findings of Pappenheimer & Robinson (48); graphs of floccule nitrogen plotted against percentage neutralisation of antitoxin with toxin gave no central linear equivalence zone, and the floccule nitrogen content at neutrality, even in floccules derived from one of Pappenheimer's preparations, considerably exceeded the figure given by Pappenheimer & Robinson [Cohn (47) has criticised this finding, suggesting that the difference is attributable to differences in the assay of the flocculating unit of antitoxin]. They then show that neutral floccules yield on digestion with pepsin at *p*H 3.2 a complex mixture of antibodies, which give numerous lines when they are allowed to diffuse against crude toxin in a diffusion column; repetition of flocculation and digestion did not greatly reduce the complexity of the mixture. Moreover, such floccule antitoxin gave a considerable precipitate with toxin-free diphtheria filtrates produced from cultures in media containing an excess of ferric iron; the material obtained by peptic digestion of this precipitate evidently contained antibodies, as it gave 6 lines in diffusion tests against crude diphtheria toxin. The supernatant from toxin-antitoxin floccule antitoxin treated with "excess iron" antigens still contained the same amount of antitoxin as before treatment but gave only two lines in a diffusion test against crude toxins. Pope *et al.*, therefore concluded that diphtheria toxin-antitoxin floccules are normally contaminated with floccules derived from other flocculating systems (some of which may be complexes of the antigenic proteolytic enzymes present in *C. diphtheriae* culture filtrates, and their antibodies), and that coflocculation of this kind might explain the discrepancies between Pappenheimer & Robinson's work and their own. Obviously, if their conclusions were correct, deductions from floccule composition about the nitrogen content of diphtheria toxin and antitoxin were equally open to suspicion, and Pope *et al.* supported their case by showing that fractionation of ultrafiltered crude diphtheria toxin preparations and subsequent purification yielded one fraction (not necessarily pure) containing 3200 Lf. per mg. nitrogen, a figure considerably higher than that of Pappenheimer & Robinson.

Lepow & Pillemer (79) have shown independently of Pope *et al.* that

electrophoretically homogeneous fractions of diphtheria toxin containing 2400 to 2600 Lf. per mg. nitrogen can be obtained but that they vary widely in their content of *C. diphtheriae* precipitinogen. Bowen (80) has adduced further evidence that the lines in diphtheria toxin-antitoxin diffusion columns are attributable to distinct flocculating systems and that purified diphtheria toxins and toxoids may contain numerous other antigens.

Later Pope & Stevens (81), by repeated treatment of crude pepsin-refined diphtheria antitoxin with pepsin-treated toxin, excess iron antigens, and a diethyleneglycol extract of diphtheria bacilli to remove nonantitoxic antibodies, were able to increase the purity of the antitoxin from 16,000 units per gm. to 211,000 units per gm; though this figure far exceeds that previously reported for floccule antitoxin [135,000, Pope & Healey (82)], they do not regard the material as pure.

They then (83) examined the optical density of mixtures of crude diphtheria toxin and this purified diphtheria antitoxin, and of toxin and other purified antitoxin samples obtained by similar means from antisera produced in horses by immunising them with flash-heated toxin. They showed that as the antitoxin was purified, the curve of optical density plotted against mixture composition changed from the broad irregular curve characteristic of crude or even of floccule antitoxin until with the purest antitoxin preparations available, it was narrow, smooth, and symmetrical about the neutral point; the purer the antitoxin the less the maximal optical density of the flocculating mixture. Moreover there was little evidence that floccules derived from highly-purified antitoxin adsorbed any antibody in the zone of antibody excess, as floccules derived from cruder antitoxin preparations undoubtedly do [Healey & Pinfield (84)]. Pope & Stevens interpret these findings as meaning that much of the floccular material produced in a mixture of crude diphtheria toxin and even partly purified antitoxin is composed of floccules derived from other antigen-antibody systems, and they even go so far as to suggest that pure diphtheria toxin and antitoxin may not flocculate at all in the absence of contaminating antigen-antibody systems. This would clearly mean that not only would pure antitoxin fail to flocculate with crude toxin, but that it ought to be possible to purify diphtheria toxin to a point at which it would not flocculate with crude antitoxin. Their crystalline diphtheria toxin indeed still flocculates with diphtheria antitoxic sera, but they do not regard it as pure (85).

There the matter rests at present, though it might be well worth while to follow up the hint given by Pope *et al.* (53) who found that the fractions obtained by Derrien precipitation from crude toxin preparations all had toxin associated with them and suggested that toxin might be adsorbed on, or form complexes with, other proteins in the filtrate. In that case the figures for the purity of toxin, for example, might very well depend on which protein complexes survived the purification process.

LITERATURE CITED

1. van Heyningen, W. E., *Bacterial Toxins* (Blackwell Scientific Publications, Oxford, England, 133 pp., 1950)

2. Glenny, A. T., Barr, M., Llewellyn-Jones, M., Dalling, T., and Ross, H. E., *J. Pathol. Bact.*, **37,** 53 (1933)
3. Oakley, C. L., Warrack, G. H., and Warren, M. E., *J. Pathol. Bact.*, **60,** 495 (1948)
4. Nelson, C. I., *J. Infectious Diseases*, **41,** 9 (1927)
5. Raynaud, M., and Second, L., *Ann. inst. Pasteur*, **77,** 316 (1949)
6. Raynaud, M., *Compt. rend.* **225,** 543 (1947)
7. van Heyningen, W. E., and Gladstone, G. P., *Brit. J. Exptl. Pathol.*, **34,** 202 (1953)
8. Llewellyn-Smith, M., *League of Nations, Bull. Health Organization*, **10,** 104 (1942–43)
9. Herbert, D., and Todd, E. W., *Biochem. J.* (*London*), **35,** 1124 (1941)
10. Howard, J. G., Wallace, K. R., and Wright, G. P. *Brit. J. Exptl. Pathol.*, **34,** 174 (1953)
11. Bosworth, T. J., and Glover, R. E. *Rept. Director Inst. Animal Pathol., Cambridge*, **4,** 79 (1934–35)
12. Bosworth, T. J., and Glover, R. E., *Proc. Roy. Soc. Med.*, **28,** 1004 (1934–35)
13. Ross, H. E., Warren, M. E., and Barnes, J. M., *J. Gen. Microbiol.*, **3,** 148 (1949)
14. Oakley, C. L. and Warrack, G. H., *J. Gen. Microbiol.*, **4,** 365 (1950)
15. Oakley, C. L., *Bull. Hyg.*, **18,** 781 (1943)
16. Howard, J. G., and Wallace, K. R., *Brit. J. Exptl. Pathol.*, **34,** 181 (1953)
17. Schnayerson, A. F., and Samuels, S. L., *J. Immunol.*, **18,** 141 (1930)
18. Glenny, A. T., *J. Pathol. Bact.*, **28,** 24 (1925)
19. Glenny, A. T., and Barr, M., *J. Pathol. Bact.*, **35,** 91 (1932)
20. Glenny, A. T., and Barr, M., *J. Pathol. Bact.*, **35,** 142 (1932)
21. Glenny, A. T., Barr, M., Ross, H. E., and Stevens, M. F., *J. Pathol. Bact.*, **35,** 495 (1932)
22. Jerne, N. K., *Acta Pathol. Microbiol. Scand.*, Suppl. 87 (1951)
23. Glenny, A. T., Pope, C. G., Waddington, H., and Wallace, U., *J. Pathol. Bact.*, **28,** 333 (1925)
24. Barr, M., and Glenny, A. T., *J. Hyg.*, **44,** 135 (1949)
25. Macfarlane, R. G., Oakley, C. L., and Anderson, C. G., *J. Path. Bact.*, **52,** 99 (1941)
26. Evans, D. G., and Prophet, A. S., *J. Gen. Microbiol.*, **4,** 360 (1950)
27. Oakley, C. L., Warrack, G. H., and Clarke, P. H., *J. Gen. Microbiol.*, **1,** 91 (1947)
28. Bernheimer, A. W., *J. Exptl. Med.*, **80,** 309 (1944)
29. Warrack, G. H., Bidwell, E., and Oakley, C. L., *J. Pathol. Bact.*, **63,** 293 (1951)
30. Dalling, T., Glenny, A. T., Mason, J. H., and O'Brien, R. A., *Brit. J. Exptl. Pathol.*, **9,** 43 (1928)
31. Glenny, A. T., Llewellyn-Jones, M., and Mason, J. H., *J. Pathol. Bact.*, **34,** 201 (1931)
32. Glotowa, E. W., Ostrowskaya, O. A., Ssilanowa, I. W., and Milaschewskaya, W. G., *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, **132,** 57 (1934)
33. Hottle, G. A., and Okono, C., *J. Bact.*, **33,** 114 (1937)
34. Oakley, C. L., and Warrack, G. H., *J. Pathol. Bact.*, **53,** 335 (1941)
35. Oakley, C. L., Warrack, G. H., and van Heyningen, W. E., *J. Pathol. Bact.*, **58,** 229 (1946)
36. Oakley, C. L., and Warrack, G. H., *J. Pathol. Bact.*, **63,** 45 (1951)
37. Bosworth, T. J., *J. Comp. Pathol. Therap.*, **53,** 245 (1943)
38. Oakley, C. L., *Brit. Med. J.*, **I,** 267 (1949)
39. Burnet, F. M., *J. Pathol. Bact.*, **32,** 717 (1929)

40. Glenny, A. T., and Stevens, M. F., *J. Pathol. Bact.*, **40,** 53 (1935)
41. Llewellyn-Smith, M., and Price, S. A., *J. Pathol. Bact.*, **47,** 361 (1938)
42. Llewellyn-Smith, M., and Price, S. A., *J. Pathol. Bact.*, **47,** 379 (1938)
43. Ramon, G., *Compt. rend. soc. biol.*, **86,** 611 (1922)
44. Glenny, A. T., and Wallace, U., *J. Pathol. Bact.*, **28,** 317 (1925)
45. Glenny, A. T., and Pope, C. G., *J. Pathol. Bact.*, **30,** 587 (1927)
46. Glenny, A. T., and Okell, C. C., *J. Pathol. Bact.*, **27,** 138 (1924)
47. Cohn, M., *Methods in Medical Research*, **5,** 301–35 (The Year Book Publishers, Inc., Chicago Ill., 394 pp., 1953)
48. Pappenheimer, A. M., Jr., and Robinson, E. S., *J. Immunol.*, **32,** 291 (1937)
49. Glenny, A. T., and Wallace, U., *J. Pathol. Bact.*, **28,** 317 (1925)
50. Glenny, A. T., *A System of Bacteriology in Relation to Medicine*, **6,** 106–93 (His Majesty's Stationery Office, London, England, 538 pp., 1931)
51. Barr, M., and Glenny, A. T., *J. Pathol. Bact.*, **47,** 27 (1938)
52. van Heyningen, W. E., and Bidwell, E., *Biochem. J.* (*London*), **42,** 140 (1948)
53. Pope, C. G., Stevens, M. F., Caspary, E. A., and Fenton, E. L., *Brit. J. Exptl. Pathol.*, **32,** 246 (1951)
54. Pope, C. G., and Stevens, M. F., *Brit. J. Exptl. Pathol.*, **34,** 56 (1953)
55. Oakley, C. L., and Fulthorpe, A. J., *J. Pathol. Bact.*, **65,** 49 (1953)
56. Ouchterlony, Ö., *Arkiv. Kemi, Mineral. Geol.*, [B] **26,** 1 (1948)
57. Ouchterlony, Ö., *Arkiv. Kemi*, **1,** 43 (1949)
58. Ouchterlony, Ö., *Arkiv. Kemi*, **1,** 55 (1949)
59. Ouchterlony, Ö., *Lancet*, **I,** 346 (1949)
60. Elek, S. D., *Brit. Med. J.*, **I,** 493 (1948)
61. Elek, S. D., *Brit. J. Exptl. Pathol.*, **30,** 484 (1949)
62. Elek, S. D., and Levy, E., *Brit. J. Exptl. Pathol.*, **31,** 358 (1950)
63. Bjorklund, B., *Proc. Soc. Exptl. Biol. Med.*, **79,** 319 (1952)
64. Ouchterlony, Ö., *Acta Pathol. Microbiol. Scand.*, **26,** 507 (1949)
65. Oudin, J., *Compt. rend.* **222,** 115 (1946)
66. Oudin, J., *Bull. soc. chim. biol.*, **29,** 140 (1947)
67. Oudin, J., *Ann. inst. Pasteur*, **75,** 30 (1948)
68. Oudin, J., *Ann. inst. Pasteur*, **75,** 109 (1948)
69. Oudin, J., *Compt. rend.* **228,** 1890 (1949)
70. Pillemer, L., Wittler, R. G., and Grossberg, D. B., *Science*, **103,** 615 (1946)
71. Pillemer, L., *J. Immunol.*, **53,** 237 (1946)
72. Lamanna, C., McElroy, O. E., and Eklund, H. W., *Science*, **103,** 613 (1946)
73. Lamanna, C., Eklund, H. W., and McElroy, O. E., *J. Bact.*, **52,** 1 (1946)
74. Abrams, A., Kegeles, G., and Hottle, G. A., *J. Biol. Chem.*, **164,** 63 (1946)
75. Lowenthal, J. P., and Lamanna, C., *Am. J. Hyg.*, **54,** 342 (1951)
76. Pappenheimer, A. M., Jr., *J. Biol. Chem.*, **120,** 543 (1937)
77. Cohn, M., and Pappenheimer, A. M., *J. Immunol.*, **63,** 291 (1949)
78. Pappenheimer, A. M., Lundgren, H. P., and Williams, H. W., *J. Exptl. Med.*, **71,** 247 (1940)
79. Lepow, I. H., and Pillemer, L., *J. Immunol.*, **69,** 1 (1952)
80. Bowen, H., *J. Immunol.*, **68,** 429 (1952)
81. Pope, C. G., and Stevens, M. F., *Brit. J. Exptl. Pathol.*, **34,** 56 (1953)
82. Pope, C. G., and Healey, M., *Brit. J. Exptl. Pathol.*, **20,** 213 (1939)
83. Pope, C. G., and Stevens, M. F., *Brit. J. Exptl. Pathol.*, **34,** 241 (1953)
84. Healey, M., and Pinfield, S., *Brit. J. Exptl. Pathol.*, **16,** 535 (1935)
85. Pope, C. G., and Stevens, M. F., *Lancet*, **II,** 1190 (1953)

THE MICROBIOLOGY OF DAIRY PRODUCTS[1,2]

JAMES J. JEZESKI

Department of Dairy Husbandry, University of Minnesota, St. Paul, Minnesota

In a discussion of this field of applied microbiology, the great variety of subject matter must be emphasized. Of importance are most types of microorganisms: bacteria (pathogenic and nonpathogenic), molds, yeasts, rickettsiae, and viruses (in particular bacteriophage). The morphology, taxonomy, physiology, and biochemical activity of organisms involved in both manufacture and deterioration of dairy products is of concern to the dairy bacteriologist. Hence it would be presumptuous to feel that all pertinent contributions to the field could be covered in a review of this scope. Emphasis will be placed on papers which have not been covered in other recent reviews and on those which deal with specific problems of current interest.

Of special interest is the excellent series of comprehensive reviews by Hiscox and co-workers (1, 2, 3) at the National Institute for Research in Dairying at Reading, England. They cover, in addition to publications dealing with dairy problems, many pertinent papers in general bacteriology and other applied fields. A review on milk-borne diseases has been presented by Smith (4). Other reviews, compiled on a national basis, which contain considerable numbers of references on dairy bacteriology include the following: United States, 1949 to 1952 (5, 6); Canada (7); New Zealand (8); Netherlands, 1947 to 1953 (9); Scandinavia, 1949 to 1952 (10); Switzerland, 1948 to 1952 (11); Spain, France, Italy, and Portugal, 1948 to 1952 (12); South Asia, 1946 to 1952 (13); and Brazil (14).

PASTEURIZATION-THERMAL DESTRUCTION

An evaluation of the effect of heat on microorganisms presupposes the control of a number of factors which influence the significance of the results. Such factors as the concentration of microorganisms, the physiological state of the test culture, the previous growth history of the culture (medium, time, and temperature of incubation), and the composition of the product being heated may affect the time-temperature relationships that describe the lethal effect of heat on bacteria. This is likewise true of the design of experimental apparatus which may affect rate of transfer of heat as well as uniformity of heating, the measurement of temperature and time (particularly under HTST[2] conditions), and the lethal effects of heating and cooling periods.

[1] The survey of literature pertaining to this review was completed in January, 1954.

[2] The following abbreviations are used in this chapter: HTST (high temperature-short time); QAC (quaternary ammonium compound); AMC (acetyl methylcarbinol); BG (2-3 butyleneglycol).

Prouty (15) presents an excellent discussion of some of these factors as related to the thermal processing of fluid dairy products. Barber (16) points out the need for conducting experiments under well controlled conditions in the evaluation of HTST pasteurization.

Pathogenic microorganisms.—Of interest is the relation of milk-borne *Coxiella burneti* to Q fever infections in man. While milk has not been incriminated in the transmission of the disease, man may nevertheless come in contact with the causative agent through raw milk. *C. burneti* may survive pasteurization under certain conditions. Huebner *et al.* (17) concluded from studies on naturally infected milk that this organism does not survive HTST pasteurization. Survival did occur in one out of four trials with the low temperature holding method in a vat not equipped with a space heater. Serological evidence was obtained for survival of *C. burneti* in 3 of 32 commercial milk samples, one of which showed a positive phosphatase test, and in one of four commercial cream samples. Lennette *et al.* (18) also obtained serological evidence for the survival of this organism in 1 of 35 samples of commercial vat-pasteurized milk and 2 of 42 samples of commercial HTST pasteurized milk. The infective vat-pasteurized sample was also weakly phosphatase positive, whereas many of the other samples received heat treatments in excess of the minimum requirements. Ransom & Huebner (19) observed that egg embryo suspensions in milk survived 63°C. for 30 min., but the concentrations used were far in excess of those which have been found in naturally infected milk. A group of English workers [Marmion *et al.* (20)] obtained evidence in a small commercial HTST plant that naturally infected milk was rendered noninfective for guinea pigs at from 154 to 156°F. for 15 sec., whereas 161.5°F. for 15 sec. was required to destroy the organism in milk artificially infected with yolk sac material. A laboratory experiment indicated that from 143.5 to 144°F. for 30 min. was required to render artificially infected milk harmless for guinea pigs; 142.7°F. for 30 min. was not sufficient. Enright *et al.* (21) have demonstrated in exacting studies that *C. burneti* suspended in sterile skim milk may survive 143°F. but not 144°F. for 30 min. These results were obtained on suspensions containing up to 100,000 guinea pig doses per ml. which is approximately ten times the maximal concentration detected in naturally infected milk.

The above papers serve to emphasize certain difficulties which prevent clear-cut conclusions on this problem. Perhaps the greatest of these difficulties is the determination of the survival of *C. burneti*. Demonstration of viable rickettsiae in chick embryos is a direct and positive procedure, but unfortunately not all strains are adapted to grow in the chick embryo. The development of complement-fixing antibodies in the guinea pig is necessarily a slow procedure, and it seems evident that heat-killed *C. burneti* can give rise to antibodies in the guinea pig; thus, at least one subpassage is necessary to provide positive evidence of survival in the original heated milk (21). Other factors which may lead to variable results include the effect of

the concentration of infectious agent in the milk on the survial time at a given temperature, the physiological state of the organism at the time of heat treatment, and previous growth history of the organism. To be specific, the lack of agreement between the results obtained by certain investigators may be attributable to the nature of the milk used, skim or whole (fat or total solids percentage may be important), as well as to the source of organisms infecting the milk (for certainly there is no reason to expect that organisms as they are shed in milk are in the same physiological state as those growing in a chick embryo). Furthermore, a suspension of *C. burneti* in yolk sac material, even though mixed uniformly in milk, will have an immediate environment of a different nature than do organisms shed directly into milk. The protective effect of the embryo tissue containing the rickettsiae, however finely it may be dispersed, may be sufficent to alter the apparent heat resistance of the organism.

Of similar concern are the results of recent studies on the heat resistance of *Brucella abortus*. Foster *et al.* (22) have determined the time-temperature relationships for the heat inactivation of *B. abortus* (strain 2308) in a series of well-designed experiments. The lethal effect of the heating-up period is shown by the fact that this strain of *B. abortus* may not be completely destroyed at 161°F. for 15 sec. when instantaneous heating is employed, but appears to be killed if preheating time of one minute occurs. Whether the use of high populations in this study (about 15 million per ml.) significantly influenced the results obtained has not been determined.

It seems evident from these experiments with the pathogens tested that there is need of further work to determine definitely the behavior of these organisms toward heat in a variety of fluid products and under both methods of pasteurization. There is little doubt that present pasteurization standards approach very closely, if they do not exceed, the conditions required to destroy larger numbers of organisms than those usually present in milk being pasteurized. Of necessity, there must be a search for strains of organisms occurring naturally, and not cultured in the laboratory for long periods of time, which may be more heat resistant than those heretofore tested, and a delineation of those factors that influence survival time under practical operating conditions in a dairy plant. The culmination of such efforts should be either the reaffirmation of our present pasteurization standards or a relatively small correction to provide the necessary safety factor to cover the possible variations in survival time which have been discussed previously.

A paper of much interest is that concerned with the survival of poliomyelitis virus in milk and cream by Kaplan & Melnick (23). Holder type pasteurization (143°F. for 30 min.) was adequate for the destruction of the virus, either from feces or infected spinal cord, in water, milk, cream, and ice cream mix. The amounts of virus present exceeded what might be expected as the maximal amount of contamination in nature. Poliomyelitis virus present in fecal material was inactivated at 160°F. for 15 sec. when sus-

pended in milk, cream, and ice cream mix. Rodent-adapted virus suspended in cream was more heat resistant, inactivation of one strain requiring 160°F. for 180 sec. However, it appears on the basis of other data presented that the recommended practice of 175°F. for 25 sec. for ice cream mix would be adequate. Byrd (24) reported that the Lansing strain of poliomyelitis virus suspended in melted ice cream was more resistant to heat than in saline. A suspension of infected mouse brain in melted ice cream was partially inactivated by exposure at 70°C. for 30 min. and completely inactivated at 75°C. for 30 min. Those results on rodent-adapted virus in which ground spinal cord served as the source of virus may be merely a reflection of the protective effect of spinal cord tissue.

Other bacteria.—Speck & Lucas (25) were able to show that added solids-not-fat increase the pasteurization requirement of chocolate milk inoculated with a test organism, *Micrococcus freudenreichii* (No. MS66). No specific effect of ingredient could be measured other than the contribution to the solids content of the product. Grosche *et al.* (26) found the pasteurization requirement of concentrated whole milk (3:1) to be only slightly higher under HTST conditions than that of regular milk despite its very high solids content. A different test organism, *Micrococcus* sp. (No. MS18s) was used in these experiments, but the fact that greatly increased solids provided only a very minor increase in pasteurization requirement was thought to be attributable to other chemical properties of the product. Ice cream mix was found to have a much higher heat requirement for the destruction of the heat resistant organism *Micrococcus* sp. (No. MS102) [Barber & Hodes (27)]. A population of from 500,000 to 1,000,000 per ml. of ice cream mix required 175°F. for 25 sec. Speck *et al.* (28) confirmed these results, using the same organism and *Microbacterium* sp. (No. 342-S-1), and concluded that the present standard of 175°F. for 25 sec. appears to be more stringent than 155°F. for 30 min. The composition of the ice cream mix did not affect the pasteurization requirement, perhaps as a consequence of the rather constant sugar-content of the various mixes studied.

Other well-designed and controlled experiments have been conducted on the heat resistance of a variety of other organisms of interest to the dairy bacteriologist. Olson, Macy & Halvorson (29) observed that the thermal death times of coliform bacteria vary over a wide range, from 5 min. to more than 150 min. at 135°F., and that the most heat resistant resembled *Escherichia coli.* Further, the temperature at which a culture of *E. coli* was grown influenced its thermal death time. Under comparable conditions, the thermal death time of *E. coli* increased from 22 min. to 74 min. at 140°F. when grown at 20°C. and 37°C. respectively. Increased populations resulted in higher thermal death times and the z[3] values of the thermal death time

[3] z value represents the increase in degrees Fahrenheit which is necessary to reduce the thermal death time tenfold; F value indicates the thermal death time of any organism at 250°F. Ball, C. O., "Thermal process time for canned food," *Natl. Research Council (U.S.), Bull.*, Pt. I, 7(37) (1923)

curves exceeded the z value of the "pasteurization curve." These authors concluded that the data provide evidence explaining in part, at least, the difference observed in bacterial destruction between the HTST and holding methods of pasteurization. White (30, 31), in thermal destruction studies on *Streptococcus lactis* and *S. faecalis*, was able to differentiate readily between the two species when the log of the number of survivors was plotted versus time of exposure at 60°C. in skim milk. The graphs so obtained were not always straight lines and may be explained in several ways. First, it is entirely possible that the lags observed in the initial stages were the result of the presence of clumps, but the decrease in slope of the curve at the later stages of exposure appears to be excellent evidence of the presence of organisms of different physiological ages having corresponding differences in heat resistance. This was true, particularly, in cultures which had been incubated for only short periods of time prior to heat exposure. Bassett & Slatter (32) studied the heat resistance of lactobacilli isolated from Swiss cheese and found that 50 of 60 cultures were killed in 16 min. or less at 150°F. and only four were killed at 160°F. for 18 sec. At 155°F., 24 sec. were required to kill the least heat-resistant organisms studied. Some of these organisms were able to survive HTST pasteurization but not the holding method. Ruyle & Sognefest (33) found an extremely heat-resistant organism identified tentatively as *Bacillus subtilis* which produced sweet curdling in canned evaporated milk. This culture survived the usual sterilization process for evaporated milk, possessed the z value of 15 in evaporated milk, and the F^3 value of 3.7 at a population of 1.5×10^6 per ml. A minimum exposure of 16.4 min. at 243°F. was required to prevent spoilage in 14 1/2 ounce cans.

Thermoduric bacteria.—The bacteria surviving pasteurization have been much studied with respect to their significance, origin, and factors affecting their numbers. The more recent of the excellent series of papers by Thomas and co-workers (34 to 37) provide excellent data on English milk supplies. Their results indicate that thermoduric counts of raw milk show very little correlation with the extent of cooling except in about 4 per cent of the samples taken, and that colony counts of thermodurics in farm milk supplies and on farm utensils were generally higher in summer and early autumn. The methylene blue test at 37°C. was considered to be a poor index of thermoduric count (faulty farm sanitation), but a better indicator of inadequate cooling methods. There was, at best, only a very broad general relationship between the methylene blue reduction time and the thermoduric colony count at 30°C. The methods used in cleaning utensils influenced the rate of accumulation of thermoduric microflora, warm water washing at 120°F. causing less increase than the use of detergent solutions at from 160 to 170°F. with no additional bactericidal treatment. The increase in thermoduric count was closely associated with formation of milk stone and the multiplication (or protection) of those bacteria in the deposit.

Nashif & Nelson (38) have found that microbacteria, chiefly *Microbacterium lacticum*, may constitute as much as half of the countable flora in

pasteurized dairy products. Relatively high numbers (1 to 2×10^6 per ml.) survive 84°C. for as long as 15 min. Alexander & Higginbottom (39) observed that HTST pasteurization produced slightly lower percentage destruction of all bacteria but that both HTST and holder type pasteurization resulted in an increase in the proportions of microbacteria and micrococci. *S. faecalis* and *S. thermophilus* also were found in about half of the samples. The most recent of the papers in the series by Galesloot (40, 41) are concerned with deterioration of pasteurized milk, mostly as the result of contamination after pasteurization.

Starter Cultures

The factors influencing the growth and acid production of dairy starters and methods for their propagation have been discussed by Babel (42) and Czulak (43). Czulak & Hammond (44) have preserved single strain starter cultures by freeze-drying and have found that good activity can be maintained on storage at room temperature for 6 to 16 months. In reviewing the tremendous volume of literature amassed on this subject, one is impressed by the wide variation in characteristics of organisms which have been isolated from typical starter cultures. Many attempts have been made to classify these organisms; conventional concepts indicate that the typical acid producers are either *S. lactis* or *S. cremoris* and that the C_4 compounds, diacetyl, AMC[2], and BG[2] responsible for flavor and aroma, as well as some volatile acids and CO_2, are produced generally by *Leuconostoc dextranicum* and *L. citrovorum*. A host of related organisms have been described which possess characteristics intermediate between the two groups and which have many characteristics of heterofermentative streptococci.

Taxonomic studies.—Some of the more recent efforts to identify these organisms have employed serological techniques for classification. Swartling (45) reported that cultures very closely resembling *S. diacetilactis*, a citric acid fermenter isolated from milk and starter cultures, belong in group N along with *S. cremoris* and *S. lactis*. Briggs (46) pointed out the difficulties encountered with nonspecific reactions and suggested procedures for preparation of specific group N precipitating sera. Further work by Briggs & Newland (47, 48) has confirmed the position of *S. cremoris*, *S. lactis*, and *S. diacetilactis* in group N. These results may be confusing from a biochemical point of view in that organisms commonly considered to be homolactic are grouped with one which possesses some heterolactic characteristics. Such an attempt at classification on the basis of the presence of common antigenic substances in the cell may have to be accepted with reservations. On the one hand, biochemical studies have shown that the homolactic or heterolactic nature of an organism may be merely a question of environment or variation in concentration of certain critical enzyme systems. On the other hand, Briggs & Newland (49) emphasized the complexities of antigenic structure of the lactic streptococci, pointing out the existence of varietal and heterol-

ogous antigens. Hirsch (50) suggests that the great variability of lactic streptococci with respect to the fermentation of lactose is evidence of their lack of complete adaptation to a milk environment, and consequently indicative that they are of a much younger biological age than other streptococci. Other bases for his hypothesis are the saprophytic nature (nonpathogenicity) of these organisms, their formation of antibiotics against other lactic organisms, and their normal habitat.

Fermentation characteristics.—It is commonly considered that starters produce lactic acid from lactose, and diacetyl, AMC[2], BG[2], volatile acids, and CO_2 from citrate. The production of the C_4 compounds by *Leuconostoc* is characterized by little or no development of acidity, whereas the citrate-fermenting streptococci are capable of producing considerable amounts of lactic acid and volatile acid in addition to the C_4 compounds. There have been recent efforts to elucidate the role of these intermediate types in the over-all starter fermentation. Swartling (45) showed that *S. diacetilactis* is able to produce C_4 compounds, volatile acids, and CO_2 from citrate; it thus resembles, and perhaps may be identical with, *S. citrophilus* described earlier. Lactose apparently could not serve as the source of the C_4 compounds. In minor cultural respects, *S. diacetilactis* resembles the enterococci, but is not serologically related to them; whereas *S. lactis* and *S. cremoris*, which belong to the same serological group as *S. diacetilactis*, appear to be quite different biochemically, since they do not usually ferment citrate. It should be noted that organisms identified as *S. lactis* and *S. cremoris* were observed to form diacetyl by Krishnaswamy & Babel (51). In these experiments, citrate was not fermented by any of the organisms studied. The complete heterogeneity of biochemical fermentation patterns in this group of bacteria leads to much confusion.

Kneteman (52) observed, in disagreement with Swartling, that *S. citrophilus* was able to produce AMC and diacetyl from sugar as well as from citrate. On this basis, this organism may be classified as truly heterofermentative, and Swartling's reluctance to consider the citrate fermenters as heterolactics may be unwarranted. Supinska-Jakubowska (53) presented some evidence that AMC production may be related to glucose concentration even in the presence of relatively large amounts of added citrate. However, the response to glucose was not nearly so great as to citrate. Swartling & Lindgren (54) have indicated that starter cultures from Sweden contain *S. lactis* or *S. cremoris* and *S. diacetilactis*, while in starters from Norway and Holland the *S. diacetilactis* may be replaced by the *Leuconostoc* species. Their results indicated, regardless of the type of starter, that diacetyl production was related to the acidity development in the culture. The important factor in diacetyl production apparently was not the species present but the over-all effect of pH on the fermentation. Bogdanov's results (55) are not in complete agreement, since he found that when *S. diacetilactis* was mixed with *S. lactis* or *L. citrovorum*, diacetyl production decreased, much more abruptly with

the latter organism. There appear to be a number of doubtful points concerning the starter fermentation as it may be influenced by the presence of these organisms. While circumstantial evidence is good, as yet we do not have unequivocal proof that all of the C_4 compounds and CO_2 come from citrate in a conventionally mixed culture containing *S. lactis* and the *Leuconostoc* species; and much less proof if citrate-fermenting streptococci are present. We are in doubt concerning the effects of various environmental factors on the relative amounts of citrate and lactose that may be utilized in the formation of flavor and aroma compounds. Finally, there is still some doubt as to the actual mechanism of formation of the C_4-compounds, although several schemes have been proposed.

Evenhuis and co-workers (56) have suggested that the formation of diacetyl is the result of a bacteriological oxidation of some decomposition product of citric acid, since the diacetyl content of soured cream did not increase on shaking with air after the bacteria had been destroyed by pasteurization, by adding excessive amounts of HCl, or by adding quaternary ammonium salts. It has been observed (57) that on shaking a starter with air, equivalent amounts of methylglyoxal and diacetyl are formed and that diacetyl formation seems to be an oxidation-reduction process taking place at high redox potential. Further work (58) indicated that if methylene blue or quinone were added to a starter after souring had occurred, much diacetyl could be formed anaerobically; these compounds were added after souring in order to prevent inhibition of the lactic-acid-producing organisms.

In further studies on diacetyl formation (59), it was concluded that diacetyl was formed from citric acid and not from lactose. Added pyruvate resulted in increased diacetyl yields whereas added acetaldehyde did not. The rather obvious proof that pyruvate is an intermediate in the formation of diacetyl, even though neither lactose nor citrate alone can give rise to diacetyl, is an interesting and challenging observation. It is evident that pyruvate *per se* is not wholly responsible since it is readily formed in the fermentation of lactose. It seems that the several theories to explain the production of acetylmethylcarbinol and diacetyl could be tested in a series of experiments utilizing the background of the above observations. The observation that butter develops diacetyl on storage is consistent with the above observations and the theory of diacetyl formation (60).

Nutrition.—The amino acid and vitamin requirements of some lactic streptococci have been studied by Anderson & Elliker (61, 62) in an attempt to evaluate the nutritional adequacy of milk as a culture medium. A great variability among strains of *S. lactis* and *S. cremoris* was observed with respect to amino acid requirements, but serine showed a consistent slight depressing effect. Its omission from a synthetic medium resulted not only in higher acid production by almost all strains but also in the growth of several organisms which would not grow in the complete medium. Evidence of a requirement for a peptide or peptide-like substance was shown by the stimulatory effect of liver fraction L and enzymatically digested milk. The

results suggested the possibility of "strepogenin" being the stimulatory factor. Evidence of some pyridoxine synthesis was also obtained, and Tween 80 was able to replace biotin. The studies on adaptations in growth factor requirements by Cheldelin & Nygaard (63), even though performed on *Lactobacillus gayoni* and *Leuconostoc mesenteroides*, possibly provide an explanation for some of the above results. The effect of heat on the nutritional properties of milk for lactic acid organisms was studied by Foster (64). He concluded that beneficial effects induced by strong heating (up to 115°C. for 15 min.) resulted from increased availability of certain nitrogenous compounds, the most likely source being casein; but overheating was found to cause deleterious effects. Peptonized milk was found to stimulate acid production in a manner similar to that observed by Anderson & Elliker. Czulak & Meanwell (65) suggested that an inhibitor capable of surviving HTST pasteurization is present in milk in winter months as evidenced by loss of activity of single strain *S. lactis* and *S. cremoris* starters. This observation is definitely related to nutritional factors in the milk, since results on synthetic media indicate that inhibited strains have different growth requirements than do uninhibited strains.

Defects attributable to starter organisms.—It has been observed that starter organisms or others with very similar characteristics have been involved in the development of a gassy defect in cheese. Galesloot (66) isolated several strains of *Betacoccus* (*Leuconostoc*) which were responsible for an early gas defect; and while not normal inhabitants of cheese starters, large numbers were detected in a starter from a factory having trouble. Gas development was most noticeable when the starter action was weak, and acid production was poor as a consequence. Overcast & Albrecht (67) traced a gas defect in cheddar cheese to the activity of *Leuconostoc citrovorum*. Several strains isolated from gassy cheese were able to produce the defect when used along with a single strain *S. lactis* culture. Czulak (68) was able to demonstrate abnormal gas formation in cheese made with *S. diacetilactis* starters. He concluded that this organism was more dangerous than the *Leuconostoc* species as a potential source of gas formation because as an active acid former *S. diacetilactis* could become dominant, whereas the *Leuconostoc*, a relatively inert organism, would not reach such large numbers. Oliver's experiments (69, 70) on the decarboxylase systems of several lactic streptococci appear to be pertinent to this problem. Cultures of these organisms in milk retained considerable decarboxylase activity after having been heated to 100°C. for 60 min. and after sterilization of the culture by $Hg(Cl)_2$ and cetrimide, a quaternary ammonium compound (QAC). The possibility of decarboxylase activity after heat treatment of milk is of considerable practical importance in the control of the defect.

Bacteriophage

The effect of bacteriophage on starter activity is one of the more serious microbiological problems in dairy technology at present. The high incidence

of phage infection in dairy plants and the seriousness of the problem have been emphasized in studies on slow starters by Smith *et al.* (71). Discussions of the characteristics and control of *S. lactis* bacteriophage have been presented by Elliker (72), Prouty (73), and Whitehead & Harkness (74). Smythe & Lightbody (75) have outlined methods and precautions necessary for the maintenance of phage-free starter cultures. A previously unreported bacteriophage specific for *S. thermophilus* has been isolated from yoghurt by Pette & Kooy (76). This same type of phage also was obtained from Swiss cheese whey by Deane *et al.* (77). Czulak (68) reported the isolation of a series of phage strains specific for *S. diacetilactis*. A strain of *S. lactis* phage differing in size from others studied was reported by Williamson & Bertaud (78). Electron microscope studies showed the particles to be about three times longer than other strains of *S. lactis* phage previously described.

The calcium requirements of *S. lactis* bacteriophage have been described further. Collins and co-workers (79) observed that calcium was required for proliferation of phage on host strains of *S. lactis* growing in a chemically defined medium. Potter & Nelson (80, 81) reported that plaque formation was stimulated when 2.5 per cent $CaCl_2$ was used as the cell diluent. Not all phage strains reacted to the same degree, but aged suspensions provided a better response than freshly prepared filtrates. On a calcium-deficient medium, minimal concentrations of 2.7×10^{-4} to 1.7×10^{-3} *M* were required to give optimal response, and it appeared that the calcium was required for proliferation rather than adsorption of phage on the host cells. Further work (82) indicated that barium, manganous, and strontium ions would replace calcium, but at higher concentrations. It was proposed that the calcium functions in the invasion of the cell by the phage. Doull & Meanwell (83) confirmed these results on calcium and applied them as the basis for developing a method for suppressing phage development during starter growth. The culture organisms were grown on a calcium-deficient medium in such a manner that the phage was diluted out of the starter on continued subculture. Addition of citrate (0.2 per cent) also resulted in phage suppression.

Graham & Potter (84) demonstrated by studies on single infected cells and by single step growth curves that burst size and latent period are variable depending on phage strain, medium, and other factors. Zehren & Whitehead (85) have attempted to explain the greater susceptibility of some starter cultures to phage action on the basis of the characteristics of the phage race involved rather than of the organisms in the culture. More rapid phage development was observed to be a function of decreased latent period and increased burst size. Two phage races capable of attacking a single organism had different burst sizes and latent periods. Overcast *et al.* (86), investigating the effect of pH on phage proliferation, found that the lag phase of phage development was lengthened at pH levels where growth of the host was retarded; proliferation failed to occur if the pH prevented growth of the host.

The data were offered as a partial explanation for starter failures occurring from apparently normal parent cultures. The results of Graham *et al.* (87) indicated that phage can exist in multiple-strain starter cultures having an apparently normal behavior. The existence of incompletely lysed sensitive strains in low numbers is offered as possible explanation; however, a delicate and constant balance between strains must be assumed. Whitehead *et al.* (88) observed strains of phage which were adsorbed and produced growth inhibition without causing any lysis. Collins' observations (89) on the nascent phenomenon where inhibition of growth may take place without multiplication may provide some explanation of Whitehead's results.

Multiple strain cultures often show an abrupt halt in acid development in the cheese vat as a result of phage action. Collins (90) demonstrated that considerable acid could be developed even though a susceptible strain of *S. lactis* made up a large proportion of the starter population. Deane & Nelson (91) offer a good explanation of the inhibition of mixed strain cultures by reason of their isolation of multiple strains of phage from samples of cheese whey. Meanwell & Symons (92) used isolates obtained from secondary growth after phage action as the source of cultures which retained resistance to phage. These resistant organisms were found to work well in the cheese plant, but the duration of their resistance could not be predicted.

The resistance of phage to chemical and physical agents is of importance from a practical point of view. Parker & Elliker (93) observed hypochlorite to be a more effect agent for destruction of phage than quaternary ammonium compounds; at 50 and 100 p.p.m. killing was more rapid with hypochlorite. Either agent was considered to be satisfactory at 200 p.p.m. (1/100 dilution of whey used as source of phage). Bennett (94) confirmed these results generally, although higher concentrations were required, probably because a 1:2 dilution of whey was used as the source of phage. Airborne phage was killed by hypochlorite aerosols containing 0.61 gm. available chlorine per 1000 cu. ft. Graham & Nelson (95), searching for possible inhibitors of lactic streptococcus bacteriophage, observed that crystal violet suppressed growth of phage at a level (1×10^{-7} *M*) permitting growth of host cells.

Prouty (96) found that desiccated lactic streptococcus phage survived at 0°C. for 42 months and at 37°C. for 72 months. These observations must be interpreted with caution, since results would be different, no doubt, under conditions of fluctuating temperature and humidity. Czulak (97) observed considerable destruction of airborne phage by ultraviolet irradiation whereas ozone (estimated 1 p.p.m.) was ineffective. The experiments were not designed, however, to give a reliable estimate of rate of destruction. The *S. thermophilus* phage reported by Deane & Nelson (91) had a heat resistance which permitted small numbers to survive 71.7°C. for periods up to 60 min. In excess of 99.9 per cent destruction was observed, but, with initial counts of above 1×10^{7} per ml., significant numbers survived the heat treatment.

Psychrophilic Bacteria

As a result of the increased time which elapses between processing and consumption of certain dairy products under modern marketing methods, organisms capable of relatively rapid growth at refrigerator temperatures have assumed greater importance. Typically of the genera *Pseudomonas*, *Alcaligenes*, and *Achromobacter*, they have been shown to influence keeping quality. It is commonly accepted that the presence of these organisms in a product is not the result of survival through pasteurization but of poor plant sanitation practices. Rogick & Burgwald (98) could not isolate psychrophiles from 4.1 ml. samples of market milk after either HTST or vat pasteurization. Watrous *et al.* (99) obtained similar results, and they indicated that the psychrophile count was a better index of postpasteurization contamination than the coliform count. Olson *et al.* (100) confirmed these findings and concluded that the absence of psychrophiles in 1 or 2 ml. quantities of milk did not necessarily ensure a long storage life. Aureomycin added to pasteurized milk (0.2 μg/ml.) did not increase keeping quality. Greene & Jezeski (101) give data on the influence of low temperatures upon the rate of growth of several typical dairy psychrophiles. They observed that decreasing the holding temperature from 10°C. to 5°C. could lengthen the keeping time as much as a decrease from 30°C to 10°C. Incubation temperatures were found to affect markedly the biochemical characteristics of the organisms studied; and, as others have found (99), routine incubation temperatures would not permit growth of two *Pseudomonas* species. A strain of *Aerobacter aerogenes* was observed to lose its ability to produce CO_2 above 32°C. and, consequently, would escape detection in liquid coliform media at routine incubation temperatures. Burgwald *et al.* (102) likewise found concentrated milk (3:1) to be affected by psychrophiles. The reason for the increased growth observed in reconstituted milk was considered to be the contamination introduced by the water used for reconstitution. Rose & McDonald (103) demonstrated that at a storage temperature of 60°F. keeping quality of concentrated milk was poor, whereas at 40°F. keeping quality was significantly increased. They indicated that the bacteriostatic effect resulting from concentration was not great and initial contamination was considered more important. On the other hand, Olson *et al.* (104) compared concentrates (3:1 and 4:1) and recombined milks at 1°C. and 7°C. and obtained data indicating that concentration has a bacteriostatic effect. Since sterile distilled water was used for recombination, the increased psychrophile growth could not be caused by contamination introduced by the water.

Psychrophiles were found to cause serious defects in cottage cheese by Parker *et al.* (105, 106, 107). The sources were believed to be the wash water and poorly sanitized equipment. The spoilage bacteria were able to reduce the diacetyl and thus destroy the typical aroma, in addition to causing slime formation, color defects, and flavor defects in the product. Hypochlorite

provided better destruction of these organisms than QAC[2], when used at concentrations of 5 to 10 p.p.m. for 15 sec. for wash water and 100 p.p.m. for 15 sec. under conditions approximating those found in equipment sanitation. Davis & Babel (108) found species of *Proteus*, *Pseudomonas*, *Aerobacter*, *Alcaligenes*, and *Achromobacter* involved in slime formation. Several cultures were resistant to 100 p.p.m. of hypochlorite for 25 sec.; all cultures tested were killed at 62.8°C. for 2.5 min. The rate of slime formation was related to storage temperature: all cultures produced slime at 21°C., while several would not produce slime at 10°C. or 4°C. The numbers of organisms used to contaminate the curd seem somewhat greater than could be expected to occur under commercial conditions, and product storage at 21°C. is uncommon. The use of 21°C. incubation probably accounts for the presence of *Proteus* sp. in the group considered to be slime-formers.

Enzyme Studies

Studies on enzyme systems of microorganisms important in dairy products have revealed some of the mechanisms responsible for changes, both desirable and undesirable, in these products. Baribo & Foster (109) found that proteolytic extracts from *Lactobacillus casei*, *Streptococcus lactis*, and *Micrococcus freudenreichii* differed from proteolytic extracts from cheddar cheese with respect to temperature and pH optima and heat resistance. They concluded that these organisms could account for only a part of the proteolytic enzymatic activity present in cheddar cheese. The endocellular proteolytic enzyme system of *S. lactis* was studied by Van der Zant & Nelson (110, 111). A pH of 7.0 was optimal for the appearance of soluble nitrogen, tyrosine and tryptophan by cultures growing in skim milk. Proteolytic activity seemed to be closely correlated with viable cell counts, and no exoenzyme was detected in the cell-free culture medium. Cell-free extracts hydrolyzed milk, casein, and lactalbumin near neutrality; at pH 5.0 activity was slight, suggesting that this enzyme system of *S. lactis* has limited activity in acid dairy products. Friedman *et al.* (112) observed that an extracellular proteolytic enzyme concentrate from *Bacterium linens* attacked casein (both α and β fractions), but not lactalbumin or β-lactoglobulin, with appreciable activity over the range of pH 5.5 to 8.8. Dacre (113) was able to detect a significant amount of tyrosine decarboxylase in only one species of lactic acid bacterium, and concluded that tyramine production is merely incidental in the cheese-ripening process. Szumski (114) found proteolytic exoenzymes from yeasts isolated from the surface of Trappist cheese to be active in a narrow range below pH 4.6, while endocellular enzymes digested casein at higher pH levels.

The lipases of dairy microorganisms likewise have been extensively studied. Nashif & Nelson (115) demonstrated that cultures of some gram-negative organisms produced lipases most active in the region of pH 7.0, and several retained considerable activity in peptone broth after being heated to 71.6°C. for 30 min. The same workers (116, 117, 118) reported that the

lipase of *Pseudomonas fragi* could be precipitated by full saturation with $(NH_4)_2SO_4$ below 7°C., required 99°C. for 20 min. for complete heat inactivation, and appeared to be adaptive in nature. Activity in cream was observed between pH 4.9 and 8.2 with an optimum between pH 5.7 and 6.6. Furthermore, over 50 per cent activity remained after cream was pasteurized at 71.5°C. for 30 min. Kester *et al.* (119) pointed out that the action of lipolytic bacteria in butter may produce concentrations of water-insoluble acids and butyric acid so high that the butter could be liable to seizure. Goldman & Rayman (120) indicated that the extent of microbial hydrolysis was a function of the amount of surface exposed to enzyme action rather than the molecular composition of the fatty substrate. The nature of the medium in which the organisms were grown influenced the amount of lipolytic activity observed. Nelson and co-workers (121, 122, 123) observed that the lipase of the mold, *Geotrichum candidum*, had an optimum activity at pH 6.0 and was most active toward natural triglycerides. The enzyme was not considered to be adaptive, and enzyme formation was not correlated with maximum mycelium formation. This organism was shown to be able to produce high concentrations of water-insoluble fatty acids in cream. Shipe's (124) studies on the lipases of *Penicillium roqueforti* and *Aspergillus niger* showed that these lipases exhibit distinct differences in substrate specificity; the pH optimum for both was between 5.0 and 5.5. Morris & Jezeski (125) presented evidence that *P. roqueforti* possesses at least two lipases: the lipolytic activity of the mycelium and of the medium in which the mold was grown differed.

It has been emphasized by a number of investigators, and rightly so, that laboratory studies on the action of enzymes must be interpreted with caution when applied to practical problems. The environment, both physical and chemical, may be far different in the natural product undergoing change, and the state of the enzyme may be changed, depending upon whether a cell-free extract or intact cell is the source of enzyme. Furthermore, the interrelationships between enzyme concentration and substrate concentrations as influenced by diffusion of enzyme, substrate, and end products may cause the rate and extent of reaction in the test tube to differ from its rate and extent in a food product.

Media and Methods

Two new milk-free agar media have been approved for the plating of milk and dairy products for official purposes (126). These media overcome the difficulties of preparing, handling, and counting plates made with a medium containing milk. Productivity of the two media closely approach that of the previously approved medium containing skim milk. Studies which led to their acceptance were completed by Buchbinder *et al.* (127, 128) and Pessin *et al.* (129, 130). Increased concentrations of yeast extract resulted in higher counts in raw milk as compared with counts in pasteurized milk (128). White (131) showed that the use of 0.1 *N* LiOH as a diluent results in de-

creased plate counts obtained on dry milk powders, probably because of the high pH of the diluent, and reconstitution of milk powders using dilution blanks heated to 50°C. results in higher counts.

Several media specific for various groups of organisms of importance in dairy bacteriology have been proposed. For enterococci, a plating medium containing citrate, azide, and ditetrazolium chloride to provide selectivity has been proposed by Reinbold *et al.* (132). Gram-negative rods do not grow on this medium, but lactobacilli may upon extended incubation. Litsky *et al.* (133) have suggested glucose azide broth and ethyl violet azide broth for the detection of enterococci in water. Likewise, several media have been suggested for the cultivation of lactobacilli. Rogosa's medium (134), while advocated for the growth of oral and fecal lactobacilli, works very well for lactobacilli of dairy origin. An outstanding feature is the lack of necessity for autoclaving, an asset in the cultivation of fastidious strains. Fabian *et al.* (135) have formulated a medium containing V-8 juice and bromcresol green which permits differentiation of lactobacilli on the basis of their strong acid production. Briggs (136) advocated the use of a medium containing tomato juice and Tween 80 for the difficultly-cultivatable species and used it as the basic medium for six physiological tests by which a large group of lactobacilli were classified (137). A simple plating method for enumeration and isolation of propionibacteria has been suggested by Kambar *et al.* (138). Essential features of the method are the use of sodium thioglycollate in the medium and an overlay of clear agar.

Morgan *et al.* (139, 140) have proposed a sequential grading procedure in connection with the microscopic examination of milk on the basis that fewer microscopic fields need be counted, in the case of samples not close to grade limits, to yield results of equal or greater precision than those attained by present standard procedures, where the number of fields to be examined is fixed by the grade limit. This statistically-founded approach has likewise been modified for enumerative purposes at grade limits of 100,000 to 200,000 clumps/ml., with a claimed saving of 20 per cent or more in the number of fields examined with no apparent sacrifice of precision.

The membrane filter appears to have some definite uses in the field of dairy bacteriology, particularly in the examination of water supplies and equipment rinse solutions [Burke (141)]. Ehrlich (142) indicated that this apparatus could be used to count coliform bacteria in milk. The centrifugation necessary to remove the organisms from milk prior to placing them on the filter pad makes this method of little use for routine examination of milk. Wüstenberg & Menke (143) also proposed a method for determining coliforms in milk by diluting the milk prior to filtration, after which the filter pad was placed on Endo agar for incubation. The chief objection to the filter, as indicated by the preliminary procedures necessary in the preparation of the filter pads, is that it is virtually impossible to pass any significant quantity of undiluted milk through the filter without plugging it.

Many pertinent recent papers, particularly those dealing with the microbiology of specific dairy product defects, the effect of antibiotics and other inhibitors on starters, and the nutritional requirements of various organisms, have not been included in this review. The selection of papers discussed has been in no sense deliberately discriminatory, but merely the result of an individual opinion as to the relative importance of problems within the field of dairy bacteriology and the pattern of organization that would best allow the most orderly treatment of the subject within the space allotted.

LITERATURE CITED

1. Hiscox, E. R., and Rowlands, A., *J. Dairy Research*, **15**, 170–217 (1947)
2. Hiscox, E. R., and Hirsch, A., *J. Dairy Research*, **18**, 207–34 (1951)
3. Hiscox, E. R., and Briggs, C. A. E., *J. Dairy Research*, **20**, 381–429 (1953)
4. Smith, J., *J. Dairy Research*, **20**, 115–26 (1953)
5. Trout, G. M., *Dairy Sci. Abstr.*, **15**, 241–68 (1953)
6. Trout, G. M., *Dairy Sci. Abstr.*, **15**, 333–62 (1953)
7. Hood, E. G., *Dairy Sci. Abstr.*, **15**, 1–22 (1953)
8. Campbell, I. L., and Dolby, R. M., *Dairy Sci. Abstr.*, **15**, 669–90 (1953)
9. Mulder, H., Hartmans, S., Stadhouders, J. J., and Van der Have, A. J., *Dairy Sci. Abstr.*, **15**, 445–60 (1953)
10. Solberg, P., and Halse, B., *Dairy Sci. Abstr.*, **14**, 647–66 (1952)
11. Kästli, P., and Schoch, W., *Dairy Sci. Abstr.*, **15**, 517–34 (1953)
12. Mocquot, G., and Bejambes, M., *Dairy Sci. Abstr.*, **15**, 81–104 (1953)
13. Sen, K. C., and Laxminarayana, H., *Dairy Sci. Abstr.*, **15**, 922–51 (1953)
14. Ballarin, O., *Dairy Sci. Abstr.*, **15**, 833–54 (1952)
15. Prouty, C. C., *J. Milk and Food Technol.*, **16**, 66–72; 76 (1953)
16. Barber, F. W., *J. Milk and Food Technol.*, **14**, 170–72; appendix, p. *ix* (1951)
17. Huebner, R. J., Jellison, W. L., Beck, M. D., and Wilcox, F. P., *Public Health Repts. (U.S.)*., **64**, 499–511 (1949)
18. Lennette, E. H., Clark, W. H., Abinanti, M. M., Brunetti, O., and Covert, J. M., *Am. J. Hyg.*, **55**, 246–53 (1952)
19. Ransom, S. E., and Huebner, R. J., *Am. J. Hyg.*, **53**, 110–19 (1951)
20. Marmion, B. P., MacCallum, F. O., Rowlands, A., and Thiel, C. C., *Monthly Bull. Ministry Health, Med. Research Council (London)*, **10**, 119–28 (1951)
21. Enright, J. B., Thomas, R. C., and Mullett, P. A., *J. Milk and Food Technol.*, **16**, 263–66 (1953)
22. Foster, H. G., Jr., Lear, S. A., and Metzger, H. J., *J. Milk and Food Technol.*, **16**, 116–20 (1953)
23. Kaplan, A. S., and Melnick, J. L., *Am. J. Public Health*, **42**, 525–34 (1952)
24. Byrd, C. L., *Am. J. Digest. Diseases*, **19**, 55–56 (1952)
25. Speck, M. L., and Lucas, H. L., *J. Dairy Sci.*, **34**, 333–41 (1951)
26. Grosche, C. A., Lucas, H. L., and Speck, M. L., *J. Dairy Sci.*, **35**, 793–99 (1952)
27. Barber, F. W., and Hodes, H. P., *Ann. Rept. N. Y. State Assoc. Milk Sanitarians, 24th Rept.*, 173–86 (1951)
28. Speck, M. L., Grosche, C. A , Lucas, H. L., Jr., and Hankin, L., *J. Dairy Sci.*, **37**, 37–44 (1954)
29. Olson, J. C., Jr., Macy, H., and Halvorson, H. O., *Minnesota Agr. Expt. Sta. Tech. Bull.*, **202** (1952)
30. White, H. R., *Proc. Soc. Appl. Bact.*, **15**, 8–14 (1952)
31. White, H. R., *J. Gen. Microbiol.*, **8**, 27–37 (1953)
32. Bassett, H. J., and Slatter, W. L., *J. Dairy Sci.*, **36**, 409–13 (1953)
33. Ruyle, E. H., and Sognefest, P., *J. Milk and Food Technol.*, **14**, 173–75 (1951)
34. Thomas, S. B., Griffiths, D. G., Ellison, D., Davies, E., Charlton, J. A., and Jones, R. G., *J. Soc. Dairy Technol.*, **4**, 245–50 (1951)
35. Thomas, S. B., Thomas, B. F., Griffiths, D. G., and Ellison, D., *J. Soc. Dairy Technol.*, **5**, 267–71 (1952)
36. Thomas, S. B., Griffiths, D. G., and Thomas, B. F., *J. Soc. Dairy Technol.*, **5**, 331–34 (1952)

37. Thomas, S. B., Thomas, B. F., and Ellison, D., *J. Soc. Dairy Technol.*, **6**, 138–44 (1953)
38. Nashif, S. A., and Nelson, F. E., *Appl. Microbiol.*, **1**, 47–52 (1953)
39. Alexander, H., and Higginbottom, C., *J. Dairy Research*, **20**, 156–76 (1953)
40. Galesloot, T. E., *Neth. Milk Dairy J.*, **7**, 1–14 (1953)
41. Galesloot, T. E., *Neth. Milk Dairy J.*, **7**, 15–40 (1953)
42. Babel, F. J., *Purdue Agr. Expt. Sta. Bull.*, **573** (1952)
43. Czulak, J., *Australian J. Dairy Technol.*, **7**, 14–20 (1952)
44. Czulak, J., and Hammond, L. A., *Australian J. Dairy Technol.*, **8**, 89–91 (1953)
45. Swartling, P. F., *J. Dairy Research*, **18**, 256–67 (1951)
46. Briggs, C. A. E., *Nature*, **169**, 455 (1952)
47. Briggs, C. A. E., and Newland, L. G. M., *J. Dairy Research*, **19**, 160–66 (1952)
48. Briggs, C. A. E., *J. Dairy Research*, **19**, 167–68 (1952)
49. Briggs, C. A. E., and Newland, L. G. M., *J. Dairy Research*, **20**, 189–97 (1953)
50. Hirsch, A., *J. Dairy Research*, **19**, 290–93 (1952)
51. Krishnaswamy, M. A., and Babel, F. J., *J. Dairy Sci.*, **34**, 374–85 (1951)
52. Kneteman, A., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **18**, 275–90 (1952)
53. Supinska-Jakubowska, J., *Acta Microbiol. Polon.*, **1**, 151–70 (1952) (English summary)
54. Swartling, P., and Lindgren, B., *Medd. Statens Mejeriförsök No. 30*, (1951)
55. Bogdanov, V. M., *Molochnaya Prom.*, **14**, 17–20 (1953). See *Chem. Abstr.*, **47**, 12678i (1953)
56. Evenhuis, N., Lerk, J., and Brouwer, H., *Neth. Milk Dairy J.*, **5**, 110–17 (1951)
57. Evenhuis, N., *Neth. Milk Dairy J.*, **6**, 137–56 (1952)
58. Evenhuis, N., *Neth. Milk Dairy J.*, **6**, 331–41 (1952)
59. Evenhuis, N., Lerk, J., and Brouwer, H., *Neth. Milk Dairy J.*, **5**, 201–28 (1951)
60. Evenhuis, N., *Neth. Milk Dairy J.*, **6**, 195–205 (1952)
61. Anderson, A. W., and Elliker, P. R., *J. Dairy Sci.*, **36**, 161–67 (1953)
62. Anderson, A. W., and Elliker, P. R., *J. Dairy Sci.*, **36**, 608–13 (1953)
63. Cheldelin, V. H., and Nygaard, A. P., *J. Bact.*, **61**, 489–95 (1951)
64. Foster, E. M., *J. Dairy Sci.*, **35**, 988–97 (1952)
65. Czulak, J., and Meanwell, L. J., *Proc. Soc. Appl. Bact.*, **14**, 1–6 (1951)
66. Galesloot, T. E., *Neth. Milk Dairy J.*, **4**, 274–87 (1950)
67. Overcast, W. W., and Albrecht, T. W., *J. Dairy Sci.*, **35**, 554–58 (1952)
68. Czulak, J., *Australian J. Appl. Sci.*, **4**, 462–68 (1953)
69. Oliver, W. H., *J. Gen. Microbiol.*, **7**, 329–34 (1952)
70. Oliver, W. H., *J. Gen. Microbiol.*, **8**, 38–44 (1953)
71. Smith, V. N., Parker, R. B., and Elliker, P. R., *J. Milk and Food Technol.*, **15**, 218–19 (1952)
72. Elliker, P. R., *J. Milk and Food Technol.*, **14**, 13–16, 44 (1951)
73. Prouty, C. C., *Proc. State Coll. Wash., Inst. Dairying, Div. Dairy Husbandry*, **22**, 59–63 (1953)
74. Whitehead, H. R., and Harkness, W. L., *Australian J. Dairy Technol.*, **7**, 3–5 (1952)
75. Smythe, V. R., and Lightbody, L. G., *Queensland Agr. J.*, **75**, 263–70 (1952)
76. Pette, J. W., and Kooy, J. S., *Neth. Milk Dairy J.*, **6**, 233–41 (1952)
77. Deane, D. D., Nelson, F. E., Ryser, F. C., and Carr, P. H., *J. Dairy Sci.*, **36**, 185–91 (1953)
78. Williamson, K. I., and Bertaud, W. S., *J. Bact.*, **61**, 643–45 (1951)

79. Collins, E. B., Nelson, F. E., and Parmelee, C. E., *J. Bact.*, **60**, 533–42 (1950)
80. Potter, N. N., and Nelson, F. E., *J. Bact.*, **64**, 105–11 (1952)
81. Potter, N. N., and Nelson, F. E., *J. Bact.*, **64**, 113–19 (1952)
82. Potter, N. N., and Nelson, F. E., *J. Bact.*, **66**, 508–16 (1953)
83. Doull, D. P., and Meanwell, L. J., *Proc. 13th Intern. Dairy Congr.*, **3**, 1114 (The Hague, Netherlands, June, 1953)
84. Graham, D. M., and Potter, N. N., *Appl. Microbiol.*, **1**, 138–42 (1953)
85. Zehren, V. L., and Whitehead, H. R., *J. Dairy Sci.*, **37**, 209–19 (1954)
86. Overcast, W. W., Nelson, F. E., and Parmelee, C. E., *J. Bact.*, **61**, 87–95 (1951)
87. Graham, D. M., Parmelee, C. E., and Nelson, F. E., *J. Dairy Sci.*, **35**, 813–22 (1952)
88. Whitehead, H. R., Hunter, G. J. E., and Cox, G. A., *J. Gen. Microbiol.*, **6**, 21–29 (1952)
89. Collins, E. B., *J. Dairy Sci.*, **35**, 371–80 (1952)
90. Collins, E. B., *J. Dairy Sci.*, **35**, 381–87 (1952)
91. Deane, D. D., and Nelson, F. E., *J. Dairy Sci.*, **35**, 668–74 (1952)
92. Meanwell, L. J., and Symons, J. M., *Proc. 13th Intern. Dairy Congr.*, **3**, 1109 (The Hague, Netherlands, June, 1953)
93. Parker, R. B., and Elliker, P. R., *J. Milk and Food Technol.*, **14**, 52–54 (1951)
94. Bennett, F. W., *Iowa State Coll. J. Sci.*, **26**, 169–70 (1952)
95. Graham, D. M., and Nelson, F. E., *J. Gen. Physiol.*, **37**, 121–38 (1953)
96. Prouty, C. C., *Appl. Microbiol.*, **1**, 250–51 (1953)
97. Czulak, J., *Proc. 13th Intern. Dairy Congr.*, **3**, 1094 (The Hague, Netherlands, June, 1953)
98. Rogick, F. A., and Burgwald, L. H., *J. Milk and Food Technol.*, **15**, 181–85 (1952)
99. Watrous, G. H., Jr., Doan, F. J., and Josephson, D. V., *Penna. State Coll. Agr. Expt. Sta. Bull., No. 551* (1952)
100. Olson, J. C., Jr., Willoughby, D. S., Thomas, E. L., and Morris, H. A., *J. Milk and Food Technol.*, **16**, 213–19 (1953)
101. Greene, V. W., and Jezeski, J. J., *Appl. Microbiol.*, **2**, 110–17 (1954)
102. Burgwald, L. H., Paxton, J. A., and Gould, I. A., *Milk Dealer*, **42**, 50, 130–34 (1952)
103. Rose, D., and McDonald, I. J., *Can. Dairy Ice Cream J.*, **32**, 38 (1953)
104. Olson, J. C., Jr., Nielsen, A. J., Thomas, E. L., and Morris, H. A., *J. Dairy Sci.*, **36**, 817–24 (1953)
105. Parker, R. B., Smith, V. N., and Elliker, P. R., *J. Dairy Sci.*, **34**, 887–93 (1951)
106. Parker, R. B., and Elliker, P. R., *J. Dairy Sci.*, **36**, 843–49 (1953)
107. Parker, R. B., Coldwell, A. L., and Elliker, P. R., *J. Milk and Food Technol.*, **16**, 136–39, 152 (1953)
108. Davis, P. A., and Babel, F. J., *J. Dairy Sci.*, **37**, 176–84 (1954)
109. Baribo, L. E., and Foster, E. M., *J. Dairy Sci.*, **35**, 149–60 (1952)
110. Van der Zant, W. C., and Nelson, F. E., *J. Dairy Sci.*, **36**, 1104–11 (1953)
111. Van der Zant, W. C., and Nelson, F. E., *J. Dairy Sci.*, **36**, 1212–22 (1953)
112. Friedman, M. E., Nelson, W. O., and Wood, W. A., *J. Dairy Sci.*, **36**, 1124–34 (1953)
113. Dacre, J. C., *J. Dairy Research*, **20**, 217–23 (1953)
114. Szumski, S. A., *Abstr. Doctoral Dissert., Penna. State Coll.*, **14**, 87–90 (1952)
115. Nashif, S. A., and Nelson, F. E., *J. Dairy Sci.*, **36**, 698–706 (1953)

116. Nashif, S. A., and Nelson, F. E., *J. Dairy Sci.*, **36**, 459–70 (1953)
117. Nashif, S. A., and Nelson, F. E., *J. Dairy Sci.*, **36**, 471–80 (1953)
118. Nashif, S. A., and Nelson, F. E., *J. Dairy Sci.*, **36**, 481–88 (1953)
119. Kester, L. T., Nelson, F. E., and Peters, I. I., *J. Dairy Sci.*, **36**, 794–98 (1953)
120. Goldman, M. L., and Rayman, M. M., *Food Research*, **17**, 326–37 (1952)
121. Nelson, W. O., *J. Dairy Sci.*, **35**, 455–62 (1952)
122. Purko, M., Nelson, W. O., and Wood, W. A., *J. Dairy Sci.*, **35**, 298–304 (1952)
123. Nelson, W. O., *J. Dairy Sci.*, **36**, 143–51 (1953)
124. Shipe, W. F., Jr., *Arch. Biochem.*, **30**, 165–79 (1951)
125. Morris, H. A., and Jezeski, J. J., *J. Dairy Sci.*, **36**, 1285–98 (1953)
126. American Public Health Association, *Standard Methods for the Analysis of Dairy Products*, 10th ed. (American Public Health Association, Inc., New York, N. Y., 345 pp , 1953)
127. Buchbinder, L., Baris, E., Alff, Y., Reynolds, E., and Dillon, E., *Pub. Health Repts. (U.S.)*, **66**, 327–40 (1951)
128. Buchbinder, L., Baris, Y., and Goldstein, L., *Am. J. Public Health*, **43**, 869–72 (1953)
129. Pessin, V., and Black, L. A., *J. Milk and Food Technol.*, **14**, 98–102 (1951)
130. Pessin, V., and Robertson, A. H., *J. Milk and Food Technol.*, **15**, 104–9 (1952)
131. White, A. H., *Sci. Agr.*, **32**, 19–25 (1952)
132. Reinbold, G. W., Swern, M., and Hussong, R. V., *J. Dairy Sci.*, **36**, 1–6 (1953)
133. Litsky, W., Mallmann, W. L., and Fifield, C. W., *Am. J. Public Health*, **43**, 873–79 (1953)
134. Rogosa, M., Mitchell, J. A., and Wiseman, R. F., *J. Bact.*, **62**, 132–33 (1951)
135. Fabian, F. W., Fulde, R. C., and Merrick, J. E., *Food Research*, **18**, 280–89 (1953)
136. Briggs, M., *J. Dairy Research*, **20**, 36–40 (1953)
137. Briggs, M., *J. Gen. Microbiol.*, **9**, 234–48 (1953)
138. Kambar, C. S., Reinbold, G. W., and Hussong, R. V., *J. Dairy Sci.*, **35**, 915–19 (1952)
139. Morgan, M. E., MacLeod, P., Anderson, E. O., and Bliss, C. I., *J. Milk and Food Technol.*, **15**, 3–7 (1952)
140. Morgan, M. E., and MacLeod, P., *J. Milk and Food Technol.*, **16**, 228–31 (1953)
141. Burke, C. P., *Food Eng.*, **25**, 47 (1953)
142. Ehrlich, R., *J. Milk and Food Technol.*, **16**, 6–8 (1953)
143. Wüstenberg, J., and Menke, P., *Milchwissenschaft*, **8**, 44–48 (1953)

FOODS AND FEEDS FROM FUNGI[1]

By F. S. Thatcher

Microbiology Section, Food and Drug Divisions, Department of National Health and Welfare, Ottawa, Canada

Current interest in the synthesis of food by microorganisms arises from three main considerations: (*a*) the critical need for food, and in particular for protein, that exists in many parts of the world; (*b*) the economic advantages of microbial elaboration of foods or vitamins from relatively inexpensive raw materials; (*c*) the reduction of the cost of disposal of fermentable factory wastes.

The reader is asked to accept for the present purpose an interpretation of the titular word "fungi" to include mycelial forms of the Eumycetes (molds), the yeasts, and bacteria. Their potential contribution to the world's food supply will be discussed with regard to the synthesis of fat and protein and of other substances of dietary value; the specific amino acid and fatty acid content of fungal proteins and fats; the nutritional value of fungal foods; their toxicity; the microorganisms studied; and the technology of production from the point of view of basic factors related to the organism, its environment, and commercial substrates.

The basis of commercial food synthesis by fungi is the high degree of efficiency of these organisms in using energy from cheap carbohydrates to convert elementary nutrients into protein and fat [Foster (78)]. The potential significance of such efficiency is evidenced by the calculation that the produce from one acre of arable land can be converted into ten times as much protein by fungi as by animals [Robinson (188)]. Yield data are tabulated by Dunn (62).

The Production of Fats by Microorganisms

Early attempts to exploit the potentialities of microbial syntheses were directed towards production of fats to alleviate shortages during the first World War. Much of the earlier literature is reviewed by Fink & Lechner (71) and others (19, 66, 127, 149, 165). Fink & Lechner describe methods for obtaining relatively high yields of fats from *Endomycopsis vernalis*. Whey with added ammonium salts was the commonest medium, and surface culture was the only method to prove successful. *Torulopsis utilis* superseded *E. vernalis* largely through the developmental studies of Fink *et al.* (70 to 74) who obtained higher yields in shorter growth periods. Specific strains of *Oidium lactis* were also selected for fat synthesis after a study of some 50 strains. Fink *et al.* was able to show that the relative amounts of fat and protein synthesized could be modified by adjustment of the C:N ratio of the medium. In general, the synthesis of protein and rate of growth were pro-

[1] The survey of the literature pertaining to this review was concluded in December, 1953.

portional to the relative amount of nitrogen in the medium. As available nitrogen became limiting, growth rates were greatly reduced and the major synthetic activity changed to the formation of fat, which appeared as globules within the older cells of the culture. This observation has been shown to be true of other fungi by several workers: *Nectaromyces rekaufii* and *Torulopsis pulcherrima* [Rippel (185)]; *Torulopsis lipofera* [Kleinzeller (125)]; *Rhodotorula glutinis* [Nilsson *et al.* (161)]; *Toluropsis utilis* and *Rhodotorula gracilis* [Enebo *et al.* (66)]; *Mucor circinelloides* and *Fusarium bulbigenum* [Bernhauer *et al.* (22 to 26)]; *R. gracilis* [Nielsen *et al.* (160)]; and an unnamed soil yeast [Starkey (213)]. Nilsson *et al.* obtained satisfactory yields (35 per cent dry weight) of fat in submerged cultures. Nord & Mull (162) obtained good yields of fat in aerated cultures of *Fusarium lini.* Kleinzellar (125) increased yields of fat up to 45 per cent with *T. lipofera* by addition of phosphates. Yields were increased up to 64 per cent [Enebo *et al.* (66)] by use of selected strains of *T. gracilis* adapted for fat synthesis and by vigorous aeration with agitation, two essentials for efficient economic synthesis by most microorganisms. By selection of strains, coupled with appropriate modification of the C:N ratio Enebo *et al.* (66) were able to obtain yeast cultures with either high or low fat content, the latter being rich in protein. The economic usefulness of the method for fat production is somewhat limited by the long growth periods required for a maximum yield of fat. Prolonged cultivation reduces the fat-coefficient (units of fat synthesized per unit of sugar metabolized).

Bernhauer and coworkers (22, 23, 24) described the relative fat-protein production of 14 different fungal species of the genera *Mucor, Fusarium, Zygorrhyncus, Phycomyces, Cladosporium, Trichoderma, Penicillium,* and *Endomyces,* and the influence upon fat production of the C:N ratio and aeration. With an N:C ratio of 1 to 20 *M. circinelloides* yielded 40 to 50 per cent protein and 5 to 10 per cent fat ; with a N:C ratio of 1 to 200, the yield figures were practically reversed. The highest yields of both protein and fat were from *M. circinelloides* and *F. bulbigenum:* fat 50.5 and 45.6 per cent, protein 40 and 45.7 per cent, respectively. Relative fat-protein production in synthetic media by several fungi were estimated by Diemair & Boresch (59).

Aristovskaya (6) isolated a *Fusarium* which utilizes sugar and dextrin to give respective fat yields of 32 and 38 per cent by surface and submerged culture. Pan *et al.* (164) obtained a yield of 64 per cent and a fat coefficient of 18 from *Rhodotorula gracilis* in deep culture using vigorous aeration by sparging. The medium consisted of molasses with added $(NH_4)_2SO_4$ and KH_2PO_4, and the quantities of these salts were critical for maximum yields. Wiley *et al.* (236) report the fat, protein, fibre, carbohydrate, B vitamins, calcium, phosphorous, and ash content of *T. utilis* grown on various spent sulphite liquors (wood pulp industry) and several industrial wastes. High vitamin yields in particular are noted. Woodbine *et al.* (237) examined fat production by 40 strains of the genera *Mucor, Aspergillus, Penicillium,* and

Fusarium. Maximum fat yield was obtained from *Aspergillus flavipes* (39.7 per cent) and *Fusarium lini* (34.6 per cent). Such yields were inferior to those obtained from *Rhodotorula* and *Chlorella* and might be economically feasible only from "low grade" carbohydrate sources obtained from wastes. A comprehensive review on the production of foods by *Chlorella pyrenoidosa* and other green algae by Burlew (34), contains much information pertinent to fungal studies.

Analyses by Hilditch & Shrivastava (108) of yeast fat indicated that it was similar to other vegetable fats except that it was relatively low in hexadecanoic acid. The components of *F. lini* were determined by Fiore (75), and of *R. gracilis* by Bass & Hospodka (13). The latter workers report marked changes in the proportions of palmitic, oleic, linoleic, linolenic, and C-20 acids produced at different temperatures. Lindquist (142, 143) describes the main protein and carbohydrate constituents of brewer's yeast and Schormüller (196) the composition of *O. lactis*.

Methods of fat extraction from mycelium were studied by Bernhauer *et al.* (25) and from yeast by Brasch (29). Fungal phosphatides have been studied by Dirr & Ruppert (60). Bernhard (20) has studied the uptake of deuterium from water in the medium and its ultimate deposition in fungal fats, while Weinhouse *et al.* (231) have reported on the incorporation of alpha- and beta-carboxyl-labelled carbon in glycine into the lipids of yeast. Other papers on fungal fats will be referred to in a later section on Nutritional Values. The results of analyses for the specific fatty acid content of fungi are tabulated by Dunn (62) and Lundin (148). Comparison with other food fats may be made by reference to Jacobs (119). Wide variability in specific fatty acid content exists among the different fungi. Similar variation within a single strain has been pointed out [Bass & Hospodka (13)].

Protein from Microorganisms

Interest in fungi as a source of protein perhaps stems from the work of Takata (219) in 1929, who showed that the mycelium of *Aspergillus oryzae* contained 38 per cent protein and was also a rich source of B vitamins. In 1932, Clutterbuck *et al.* (46) isolated from yeast a protein fraction in which arginine, histidine, cystine, and lysine were identified. The early studies were reviewed in 1934 by Skinner (204), who also produced mycelium of *Penicillium flavo-glaucum* with a high protein content. The publications of Bernhauer and his co-workers (22, 23, 24) and those of Bernhard (20, 21) concerning the influence of the C:N ratio on the relative production of protein and fat are important guides. The species receiving most attention were *Saccharomyces cerevisiae*, *T. utilis*, and *R. gracilis*. The earlier studies on protein production by *T. utilis* were reviewed by Sperber (209), and of yeasts by Bunker (33). Later reviews are those by Dunn (62), Vinson (227), and Lee (132). Species studied during the search for suitable organisms for protein production are listed in Table I.

The production of food yeast for protein is now common. Surplus yeast

TABLE I

SPECIES STUDIED FOR PROTEIN PRODUCTION

Genus	Species	Source references
Aspergillus	*oryzae*	Takata (219); Wooley *et al.* (238); Suminoe *et al.* (214).
	sydowi	Skinner *et al.* (202); Wooley *et al.* (236).
	fischeri	Skinner *et al.* (203); Wooley *et al.* (238).
	nidulans	Skinner & Muller (205).
Penicillium	*flavo-glaucum*	Skinner & Muller (205); Skinner (204).
	roquefortii	Skinner & Muller (205).
	chrysogenum	Wooley *et al.* (238).
	griseofulvum	Smithies (207).
	spp.	Bernhauer *et al.* (23); Grosser & Bernhauer (96).
Fusarium	spp.	Vinson *et al.* (228); Bernhauer & Rauch (42); Heines & Nord (107); Nord & Mull (162); Aristovskaya (6).
Endomycopsis	spp.	Chiao & Peterson (42)
Endomyces	spp.	Agarwal *et al.* (3); Bernhauer & Rauch (22).
Saccharomyces	spp.	Sure (215, 216); Chiao & Peterson (42); Agarwal & Peterson (3); Demmler (57); Springer (210); Muller (157); Porges *et al.* (176); Györgi *et al.* (97); McLean & Beveridge (153); Reinartz & Lafos (183); Laufer *et al.* (130); Singh *et al.* (201); Harris *et al.* (100); White & Munns (234); Komarova (126); Campbell (35); Iwata (118); Marcilla & Hidalgo (151); Kunze (128).
Rhodotorula	spp.	Chiao & Peterson (42); Nilsson *et al.* (161); Sandegren *et al.* (193).
Torula	spp.	Agarwal *et al.* (3); Graham *et al.* (95); Porges *et al.* (176); Schormüller (196); Pavcek (167); Harris *et al.* (102, 104); Diemar & Neu (58).
Torulopsis	spp.	Ehrensvard (64); Singh *et al.* (201); Fink & Lechner (71); Wiley *et al.* (236).
Mucor	spp.	Bernhauer & Rauch (22).

from breweries is one source [Dunn (62)]. Cane and beet molasses, whey, sulphite liquor, wood sugar, and various agricultural wastes are the substrates commonly fermented. Recent developments will be discussed in the sections dealing with technology and substrates.

Kapfhammer (122) reported an extensive analysis of protein from the mycelium of *Fusarium* species. The content of N, S, P and of arginine, proline, hydroxyproline, lysine, histidine, tyrosine, and leucine were determined. Nielsen *et al.* (160) made analyses of amino acids of protein-rich and protein-poor cells of *R. gracilis*. The amino acids found by paper chromatography were α- and β-alanine, arginine, aspartic acid, glutamic acid, glycine, *iso*-leucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, and histidine. The presence of the 10 "essential" amino acids [Rose (190)] should be noted. Differences between the amino acid content of the protein from the two sources were minor. The low-protein yeast lacked β-alanine but contained greater amounts of α-alanine and glutamic acid. It was suggested that the accumulation of the two latter amino-acids in the slowly metabolising "low-protein" yeast could result from a reduced rate of transamination. For recent reviews of protein metabolism the reader is referred to Fruton & Simonds (80) and Fruton (81).

Amino acid analyses have been reported for various microbial species, including a strain of *P. chrysogenum* used for penicillin production [Yokoyama (239)], six species of the Enterobacteriaceae, *Staphylococcus aureus Pseudomonas aeruginosa* and *Streptococcus hemolyticus* [Mondolfo & Hounie (155)], and *Mycobacterium tuberculosis* [Eguchi (63)]. Fowden (82) found the amino acid contents of *Chlorella*, brewer's yeast, and the leaves of green plants to be similar, except that yeast and *Chlorella* have a much higher proportion of the monoamino, monocarboxy amino acids and of cystine than do leaves.

Ehrensvard (64) commented upon the remarkable adaptability of *T. utilis* to a diversity of substrates. The diversity of substrates available for the growth of yeast is also referred to by Barton-Wright (17), who pointed out that, even though brewer's yeast could synthesize all its nitrogen requirements from ammonium salts, improved growth could be obtained by the addition of polypeptides such as those in wort. In particular, polypeptides increased the rate of protein synthesis in top yeast. Synthesis of amino acids in yeast during early stages of the growth cycle would seem to be correlated with the amount and nature of amino acids in the medium, yeast assimilating the simpler aliphatic amino acids before the aromatic ones. This was offered as further evidence that amino acids are assimilated as such without need for prior deamination (18). Lindquist (143, 144) has shown that protein content of "top yeast" is much greater than of "bottom yeast" and is more readily extractable.

Smithies (207) reports protein, fat, and carbohydrate of *P. griseofulvum* constituted 12, 35, and 15 per cent of the dry mycelium weight, respectively. The nutrient requirement for improved protein synthesis of *O. lactis* was studied by Fabel & Henssler (68). Maximum yields were obtained from a mixture of equal amounts of glucose and galactose with $(NH_4)_2SO_4$ as the nitrogen source. From 50 to 55 per cent protein was obtained from cells grown in a medium containing 1.3 to 1.4 per cent sugar, nitrogen equivalent to

1,000 mg. N/1. and 400 mg./1 of P_2O_5. White & Munns (234) have shown that in addition to sugars, yeasts are able to to assimilate ethyl alcohol and pyruvic and lactic acids, and that, with vigorous aeration, up to 9 per cent of the yeast yield from molasses originates from carbon sources other than fermentable sugars. Agarwal *et al.* (3) showed that nonsugar compounds could be used by *S. cerevisiae* to the extent of 21 per cent of total carbon intake, *T. utilis* to 37 per cent, and *Candida arborea* to 35 per cent. The sulphur metabolism of *T. gracilis* is being studied by Sandegren *et al.* (193).

The Nutritional Value of Fungus Foods

The nutritional value of fungi has been assessed by many workers. Braude (30) in 1942 cited 113 papers in a review of the use of yeast as fodder for cattle. The conclusion drawn was that dried yeast is a valuable foodstuff usually being rich in most essential amino acids and in B vitamins. Carter & Phillips (40) and Loesecke (146) also made comprehensive reviews of the literature on the nutritive value of yeast. Their conclusions are in general accord with those of Braude (30). A summary of opinions expressed by Lembke with regard to the value of mycelia from various fungi as a source of human food may be found in Robinson (188) or Saeman *et al.* (191).The health of human subjects fed the dried mycelium of *Fusarium*, *Candida*, *Oidium*, *Endomyces*, and *Rhizopus* was better than that of the control population on a war-time diet. *Fusarium* gave the most satisfactory results. *Fusarium* and *Rhizopus* were each reported to contain high levels of cystine, methionine, and glutathione in relation to yeast.

Feeding trials by Takata (219) showed that the dried mycelium of *A. oryzae* served as a sole source of protein for rats. Skinner (204) determined the nutritional value of proteins from *P. flavo-glaucum* and showed that cystine was present in limiting quantities. Skinner & Muller (205) later showed that the proteins from seven other fungi were deficient in cystine and methionine. Rats fed these fungi as a sole source of protein grew normally when 0.25 per cent of cystine or methionine was added to the diet. This is in accord with later work by Brunner (32) who concluded that the amounts of cystine in six different types of yeast were too low to allow their use as sole sources of protein. Cystine deficiency was suggested to be of dietary significance only when methionine was also deficient. The mycelium of *A. sydowi* was shown to be deficient as a source of protein for chicks by Gorcica *et al.* (91, 92) but was a useful feed supplement in association with whole wheat or corn gluten. This fungus served in the diet of chicks as an effective source of thiamine at 10 per cent level and of riboflavin at 1 per cent. Vinson *et al.* (228,) concluded that the dried mycelium of *F. lini* and *F. graminearum* was a more satisfactory source of protein than brewer's yeast, provided that supplements of B vitamins were included in the diet. Adriano (2) demonstrated the high nutritional value of protein from 33 speciesof common Philippine mushrooms. Lintzel (141) made a comparable study with common European edible species and concluded that their protein was approximately equal in value

to that of meat for human food. Johnson (120) offers a brief review of the use of yeasts for food.

Brewer's yeast (strain K) used as a sole source of protein and B vitamins was reported by Sure to produce "excellent growth, reproduction and lactation" in rats over four generations (215). Three other strains of yeast tested for shorter periods were also said to serve as very good sources of protein. Sure & House (217) determined the biological values of brewer's yeast and of several strains of food yeast by nitrogen balance experiments in rats. Cannon (36, 38) has tabulated the comparative nutritive values of proteins from several sources. Yeast was found to cause better weight gains than soya protein in rats but was inferior to lactalbumin or casein. Protein from several strains of *Saccharomyces* was more efficiently used than the protein from *T. utilis*. Seeley *et al.* (198) showed that the dietetic value of white bread to weanling rats was considerably enhanced by addition of from 1 to 3 per cent dried yeast. B vitamins were thought to be responsible. Carpenter (39) concludes that the proteins of soya, wheat, and oats were all dietetically superior to yeast, and, in turn, all these proteins were inferior to meat, fish, and casein. Harris *et al.* described rat feeding trials with six different species of yeast grown on wood hydrolysate, comparing them with sulphite yeast, brewer's yeast, *F. lini*, soya meal, and casein. Addition of methionine to all microorganisms and to soya meal was necessary in order to provide the same dietetic efficiency as casein (104). Chiao & Petersen (42) have made quantitative determinations of methionine and cystine in 20 yeasts selected from 10 genera. The methionine content ranged from 0.17 per cent in *Endomycopsis fibuliger* to 1.0 per cent in *R. gracilis;* and the cystine content from 0.19 per cent in *Debaryomyces matruchoti* to 0.58 per cent in *Hansenula saturnus*. The higher values for both these critical amino acids could render the fungi that produce them of more than usual value as food adjuncts. As Flodin (76) points out, the amino acids most needed for improvement of the human diet are lysine, methionine, threonine, and tryptophan. If wheat is the major source of protein, addition of lysine alone would provide a balanced amino acid intake. For rice, additions of lysine and threonine are necessary; for maize, lysine and tryptophan; for roots and legumes, methionine, lysine or both and tryptophan. It is suggested that the effective supply of food could be increased to from 50 to 100 per cent by addition of the requisite amino acid supplements.

Values for the "10 essential" amino acids taken from nine sources and calculated as percentage of dry weight of fungal protein are listed in Table II with range values preceding the average, and are compared with values for meat, bread, and milk. The figures in Table II are based on the following assumed values for the average percentage of protein (dry weight) in fungi, meat, bread and milk respectively, 47 (calculated from 11 sources) 20, 9, and 3.5 [Sahyun (192)]. The foregoing figures, compared with the minimal human adult requirement (192), suggest that to satisfy the minimal daily requirements for the three amino acids which are most commonly dietet-

ically deficient, the amounts of yeast required would be 70, 255, and 105-gm. respectively for lysine, methionine, and tryptophan.

TABLE II

PERCENTAGE COMPOSITION OF AMINO ACIDS IN VARIOUS PROTEINS

	Fungi		Meat	Bread	Milk
	Range	Average		Average	
Arginine	1.5 to 10.0	4.3	7.5	4.0	4.28
Histidine	1.3 to 7.0	2.98	2.1	2.0	2.57
Isoleucine	2.1 to 6.0	3.52	3.3	3.5	4.28
Leucine	3.3 to 7.4	4.46	12.5	12.0	16.28
Lysine	3.0 to 10.0	5.53	8.3	2.0	7.43
Methionine	1.0 to 2.7	1.6	4.2	4.0	4.0
Phenylalanine	1.0 to 4.5	2.5	4.6	5.5	5.71
Threonine	1.7 to 6.0	3.34	4.6	2.5	4.57
Tryptophan	0.3 to 1.6	0.74	1.3	1.0	1.71
Valine	2.5 to 7.0	4.0	3.3	3.0	5.43

Toxicity of microbial proteins.—Wooley *et al.* (238) have shown that mycelia of *A. sydowi*, *A. oryzae*, *A. fischeri*, and *P. chrysogenum* were all toxic to rats when they constituted 50 per cent of the total food intake. This toxicity was overcome if the diet was supplemented with casein or with yeast. Hoch & Fink (111) described an hepatic necrosis that developed in rats fed yeast as a sole source of protein. The necrosis could be prevented by feeding additional cystine or methionine. Himsworth & Lindan (109) and György & Goldblatt (97) confirmed the prophylactic effect of cystine and methionine on dietetic hepatic necrosis, and presented data to suggest that the cystine-methionine deficiency was not associated with the development of hepatic necrosis unless tocopherol was also deficient in the diet. Shaefer *et al.* (199) showed that rats deficient in Vitamin B_{12} have an increased requirement for methionine which may be significant with regard to the previous findings. György and co-workers (98) made further study of the dietetic hepatic necrosis syndrome. Heat-killed American brewer's yeast caused no necrosis in rats; living British bakers' yeast consistently caused characteristic necrosis. Analysis of the two yeasts did not suggest that lack of cystine or of α-tocopherol was responsible for the induction of necrosis, though addition of either substance to the diet tended to reduce the degree of liver damage. A toxic substance present in the living yeast was postulated. Lindan & Work (136) confirmed the relative capacity of the two types of yeast to cause hepatic necrosis but showed that in terms of the normal amino acid requirement of rats both yeasts were deficient not only in cystine, and methionine, but also in phenylalanine, lysine, and leucine. Lindan & Work (137) could not relate the nutritional inadequacy of yeast protein to lack of

the S-amino acids alone and suggested an imbalance in the ratio of S-containing to other amino acids was involved. Almquist (4, 5) presented an extensive review of the toxicity of S-amino acids and of lysine and showed that the presence of an excess of specific amino acids in a diet could cause various detrimental effects. These were thought to arise from an imbalance in the required proportions. There is an extensive review by Flodin (76) on the significance of balanced availability of amino acids.

McLean & Beveridge (153) reported that when yeast was supplied as sole source of protein to rats, dietary levels of from 5 to 18 per cent caused severe hepatic necrosis and death, but at levels above 30 per cent no necrosis was found. Casein, at 3 to 4 per cent levels was also shown to induce a low degree of necrosis, and if fat intake was increased, a concomitant necrogenesis was noted. With a fat-free diet, no necrosis was associated with any of the proteins. These workers concluded that neither a specific deficiency of cystine, methionine, and α-tocopherol, nor the presence of a toxic substance in bakers' yeast could explain adequately the cause of dietetic hepatic necrosis. Subsequent papers by Lindan & Work seem to lean towards this view (137, 138). Reinhardt (184) has since shown that hepatic necrosis of similar pathology to that of the dietetic syndrome can be produced by injection of distilled water into the liver; he has suggested that perhaps the various expressions of dietetic necrosis may be basically caused by disturbance of water equilibria or of permeability within the liver tissues. Schwartz, however, has postulated the presence of a new growth factor which may be involved in the metabolism of cystine and tocopherol in prevention of dietary hepatic necrosis (197). Carter & Phillips (40) and Dunn (62) refer to a possible objection to yeast for human feeding. High intake may cause excess uric acid in the blood.

No reports have been found of toxicity arising from microbial fats. Damm (52) states that the fat from *Fusarium* is interchangeable in practice with peanut and olive oils. Brasch (29) on the other hand points out that yeast fats have a high proportion of unsaturated fatty acids which is a commercial disadvantage in processing. The reviews by Piskur (172); Lundin (148), and the text by Christensen (44) refer to the positive edibility of fungal fats.

Other Fungal Substances of Nutritional Value

Carbohydrates.—Carbohydrates such as glycogen may comprise up to 30 per cent of dry yeast, yeast glycogen being similar to that of animal origin. Other polysaccharides may predominate in other fungi, while some, such as a specific one found in *P. chrysogenum* (156) and formed of mannitol, erythritol, and glycerol, are of particular interest. Neuberg (159) has reviewed the carbohydrates of yeast and other fungi.

Ergosterol.—Commercial interest has been directed to fungi as sources of ergosterol to be used for its antirachitic activity as a precursor to vitamin D_2. Ergosterol production varies from 0.1 to 1.70 per cent in different species

and strains [Puress *et al.* (179, 180); Savard & Grant (194); Asano *et al.* (12); Fiore (75)] with a maximum yield of 32.9 mg. for 100 ml. of medium being reported for *A. sydowi* (180). A process for increasing the ergosterol content of yeast is described by Reinartz & Lafos (183). Yeast is an economic source of ergosterol from which its irradiated product calciferol is prepared.

Vitamin B_{12}.—Originally extracted from yeasts, vitamin B_{12} is now principally obtained as a by-product from the production of streptomycin by *Streptomyces griseus*, though the mycelium of other antibiotic producing fungi is also used. A method was recently described by Hall *et al.* (99) whereby a yield of from 1.5 μg. to 3 μg. of vitamin B_{12} per ml. of medium may be produced from *S. olivaceous* grown in submerged aerobic culture. Darken (55) describes fermentation processes to produce B_{12} from *Flavobacterium devorans*, *Bacillus megatherium*, and *Clostridium tetanomorphum*. Vitamin B_{12} has also been demonstrated in sewage sludge [Hoover (112); Schendel & Johnson (195)]. These last workers report that dried activated sewage sludge fed to young pigs at the rate of 2 per cent of the pig's diet, provided a satisfactory source of vitamin B_{12} without causing any harmful effects. Some 300 papers on vitamin B_{12} were reviewed by Smith (206) in 1951, and a later review by Darken (55) on the microbial production of vitamin B_{12} carried 155 other references. For a treatment of the role of vitamin B_{12} as the "animal protein factor," the reader is referred to a paper by Coates (47).

Antibiotics.—Reviews of the diverse aspects of the value of the antibiotics themselves in nutrition have been published recently by Braude *et al.* (31) and by Jukes & Williams (121).

Carotenes (vitamin A).—Several carotenes present in a number of microorganisms have been shown to be effective precursors to vitamin A. Porter (177) reviews the earlier work on microbial carotenes. Goodwin and his coworkers (86, 89, 90) have recently described the production of carotenoids by fungi, notably *Phycomyces blakesleeanus*. The mechanism of carotenoid synthesis in *Sarcina lutea* and *P. blakesleeanus* is discussed by Arnaki & Stary (7). The subject has been reviewed by Goodwin (87, 88).

Riboflavin.—The value of yeasts as a source of the "B-complex" is treated in the reviews by Braude (30), Carter & Phillips (40), and Loesecke (146). Commercial interest has been directed particularly to vitamin B_2 or riboflavin, which is now produced in very large quantity by submerged culture technique. A United States patent issued to Tanner *et al.* (221) describes the production of riboflavin from *Ashbya gossypii*, with yields of from 20 to 40,000 μg. of riboflavin per gram of dry weight. Aerated cultures yield from 300 to 400 μg. per ml. of the medium, which contains glucose, peptone, hydrolyzed animal tankage, and corn-steep liquor. Levine *et al.* (133) discuss the production of riboflavin by the yeasts *Candida guilliermondii* and *C. flareri*, which were said to have an advantage in that exacting precaution to maintain asepsis is not necessary. The maximum potency obtained was 325 μg./ml. Leviton & Whittier (134) have enriched the riboflavin content of whey to 200 μg. of riboflavin per l. by fermenting with *A. gossypii*.

Pfeifer *et al.* (170) obtained normal yields of from 500 to 600 μg./ml., and a maximum of 848 μg. with *Ashbya*. They presented an extensive analysis of the many factors which influence yields, together with a flow sheet of a commercial plant. An excellent review of the subject is provided by Pridham (178) who reports that the organisms currently preferred for the commercial production of riboflavin are *A. gossypii* and *Eremothecium ashbyi*. Pridham also refers to production of this vitamin by *Saccharomyces* and *Torulopsis*, by the *Clostridium* species used in the butanol-acetone fermentations, by *Aerobacter aerogenes*, and by *Azotobacter*. This last is of particular interest, since *Azotobacter* can be grown and can produce riboflavin at the expense of atmospheric nitrogen. From 10 to 11 lb. of *Azotobacter* cells can be obtained from 300 l. of medium in 32 hr., with a yield of 350 μg. of riboflavin per gm. of dry weight [Lee & Burris (131)].

Nucleic acids.—A recent paper by Laufer *et al.* (130) shows that nucleic acid may be extracted from yeast in quantities amounting to 6 per cent of the dry weight. In addition, nucleotides, nucleosides, and their products by enzymic conversion, phosphorylated esters and sugar phosphates, as well as glutathione and the components of cozymase are also recoverable in sufficient quantity to render the yeast cell useful as a source of these compounds for investigational or for therapeutic purposes.

Microbial enzymes.—In addition to the direct use of microbial cells or of their by-products as food the food industry makes frequent use of microbial enzymes. Fungal amylases have long been used for the conversion of starch to fermentable sugars. An account of the use of amylases from *A. oryzae* in breadmaking is given by Reed (182), while a review of the microbial production of enzymes and an interesting account of their industrial applications is provided by Smythe (208). The production and use of amyloclastic, proteolytic, and pectic enzymes from bacteria, yeasts, and other fungi are discussed. Tauber's text (222) should also be consulted.

Foods from Bacteria

Very recently Roberts has pointed to the food potentialities latent in the bacteria (186, 187). Vigorously aerated cultures of *Escherichia coli* using crude glucose with ammonium salts as nutrients produced yields of 24 billion cells per ml. in 9 hr. at 39°C. When heat-killed, the dried cells provided a highly satisfactory protein supplement for rats and chicks. *E. coli* protein could substitute for animal protein and the following highly interesting conclusion was drawn: " . . . the fact that the peculiarly exacting demands of the chick can be met, warrants the prophecy that diets based on *E. coli* protein for other farm animals could be evolved with little difficulty." Anderson and co-workers (8, 9), studying the influence of antibiotics in nutrition, have reported improved growth in chicks after feeding *E. coli* cultures.

The work of Roberts suggests that the proteins of *E. coli* are not deficient in methionine or cystine, as are the proteins of a number of fungi.

Mondolfo & Hounie (155) have shown that these amino acids commonly "deficient" in yeast and fungal cells are present in larger amounts in a number of bacteria, while Eguchi (63) has reported that the methionine content of *Mycobacterium tuberculosis* is of some ten times that normally found in yeasts and fungi [see earlier comment re Chiao (42)]. It seems reasonable to suggest that the deficient amino acids in yeasts could be supplemented from bacterial sources. Indeed, a symbiotic action similar to that suggested has already been tried in the joint use of yeasts and lactic acid bacteria as a means of increasing the food value of whey [Graham *et al.* (95)], while Agarwal has suggested symbiotic culture as a means of most efficient use of the substrate (3). Similarly, since the bacteria present in the rumen are considered to supply a large part of the protein and vitamin requirements of ruminant animals, further exploration of the potentialities of these organisms for the production of food and feeds either in pure-culture or in their natural symbiotic associations might well be undertaken. Heald (105) has already grown many isolates from the rumen in liquid media designed to simulate rumen fluid. In the rumen, hemicelluloses, xylose, and uronic acids, are actively fermented. McNaught (154) has demonstrated fermentation of starch, inulin, tri- and disaccharides, hexoses, and pentoses by the rumen microflora.

Substrates for Microbial Food Production

Whey and dairy wastes.—Early studies on the production of fat- and protein-yeasts were based upon the use of whey. Various lactose-fermenting yeasts grown on whey are described by Muller (157). *O. lactis* was selected by Fabel (67) for the production of feed yeasts from whey in submerged culture. Cell synthesis accounted for 18 per cent of the dry matter after fermentation, of which 50 per cent was protein. The status of the German yeast industry in 1945 was reviewed by Pavcek (167). Springer (210) reports an analysis of pressed yeast prepared from whey in which dry matter contained 54 per cent crude protein. Protein and vitamin values were said to compare favourably with yeasts from other sources. A number of the B vitamins, vitamin C, and ergosterol occurred at levels which would provide useful supplementary sources. Demmler (57) adapted for use with whey the Waldorf process, employing the anti-foaming, aerated, continuous culture device originally described for the production of yeasts from sulphite wastes. However, the author considered the process to be uneconomic in spite of protein yields as high as 52 per cent. Porges *et al.* (176) used *Saccharomyces fragilis* to remove lactose and soluble protein from whey, to which 1 per cent skim-milk had been added, for subsequent use as cattle feed. In order to make effective use of larger amounts of skim milk, additions of ammonium sulphate to the whey were necessary. Fermentation in this manner as a means for minimizing the waste disposal problem from cheese factories was not considered practicable by these workers since the process was costly and the

fermented medium could still undergo putrefaction. Hoover (113) has reviewed recent work on fermentation of dairy wastes for the purpose of reducing B.O.D. A later series of papers by Graham and co-workers (93, 94, 95) describes the synthesis of proteins and vitamins from whey by methods that provide a stabilized, marketable feed adjunct and satisfactory whey disposal. The four yeasts tested, one of which was a thermophilic strain of *T. utilis*, were all used in conjunction with a lactic culture: *Streptococcus lactis* with the mesophilic yeasts and the thermophile *Lactobacillus bulgaricus* with *T. utilis*. *Torula cremoris* was the yeast selected for further study, since it grew vigorously when aerated and preferred lactose as a carbon source.

Sulphite liquor.—In Germany more yeast is produced from sulphite liquor than from whey [Pavcek, (167)]. *Torula* and *Candida* species were adapted to this substrate by virtue of their ability to use pentoses [Brahmer, (28)]. A Canadian plant that has produced feed yeast from sulphite liquor since 1935 is described by Campbell (35). Inskeep *et al.* (117) describe the production of food yeast from sulphite liquor in a modern United States plant where *T. utilis* is used to provide a dried product of high vitamin content and containing 50 per cent protein, said to compare favourably with milk and meat in its amino acid composition. These authors point out that alcoholic fermentation of the hexoses in sulphite liquor removes only about 50 per cent of B.O.D., whereas the continuous fermentation which they describe removes hexoses, pentoses, uronic acid, and a number of the volatile acids, greatly facilitating disposal of the waste.

Wood hydrolysates.—Peterson *et al.* (169) describe in detail the methods they have used for the production of yeast-protein from the acid hydrolysate of wood enriched with urea and phosphates. Of the nine different yeasts studied, strains of *T. utilis*, *Candida tropicalis* and of an unidentified yeast gave the best yields: 90 per cent of the sugar was fermented, with 40 per cent recovery of cell substance in terms of the total reducing sugar present in the medium. A modified process for "speedier" acid hydrolysis of a variety of woods was described by Harris & Johnson (101). This process yields sugars of which 50 to 85 per cent are fermentable by *Saccharomyces* species, 90 to 95 per cent by species of *Torula*, *Monilia*, or *Candida*, and 95 to 96 per cent by *Clostridium butylicum* or *Aerobacter aerogenes*. Heines & Nord (107) described a fermentation of wood hydrolysate by *F. lini* in conjunction with yeast. The biochemistry and industrial use of the genus *Fusarium* were reviewed by Nord & Mull (162). Kurth (129) selected *T. utilis* from a group of three test yeasts for the fermentation of "wood sugar stillage" (the "still-waste liquor" remaining after alcoholic fermentation). Harris *et al.* (103) produced fodder yeast from wood sugar by use of the Waldorf fermenter, *Torula* being the most suitable organism. Harris & Johnson (101) describe a fodder yeast factory that produces 8,000 lb. of yeast per day from the wastes of a wood-pulp plant, a yield of from 150 to 200 lb. per ton of pulp manufactured. The use of two strains of *T. utilis* and one of *O. lactis* for the

production of food yeasts from beechwood sulfite liquor is reported by Diemair & Neu (58). The production of yeast from hydrolyzed wood in Japan is reported by Iwata (118).

Molasses.—Thaysen & Morris (224) report successful yields of protein from a polyploid yeast, *T. utilis* var. *major*, adapted for commercial production from molasses in the West Indies [Thaysen (225)]. Other authors who have made contributions to the use of molasses as substrate for fungal synthesis are Singh *et al.* (201), Agarwal & Peterson (3), Wiley *et al.* (236), Komarova (126), McGinnis (152) Chiao & Peterson (42), Pan *et al.* (164), and Marcilla & Hidalgo (151). Ammonium salts and phosphates are usually added to the molasses medium. A review of the use of molasses in the commercial production of *R. gracilis* for food yeast is presented by Dankwerts & Sellers (53).

Agricultural wastes.—Humfeld (115) and Sugihara (116) described production of mycelium of the common edible mushroom, *Agaricus campestris*, in submerged culture. A product (35 per cent protein, 23 per cent fat) similar in flavour and composition to commercial mushrooms was obtained from various waste products including asparagus butt juice and pressed-juice from pear waste. Humfeld suggests that the technique would be applicable to the culture of many related higher fungi. Arrazola, Marino & Cia (11) describe a new food-yeast industry, based on the fermentation by *T. utilis* var. *magna* of the tuberous rhizomes of *Asphodelus albus*. Miscellaneous native plant materials were also shown to provide suitable substrates for yeast fermentation [Arrazola (10)]. Suminoe & Ketsu (214) describe a method of forming a feed supplement for cows and pigs by growing *A. oryzae* on a mixture consisting of 10 parts of dried tree leaves and 3 parts of human, cow, or pig faeces. Other carbohydrate sources that have been fermented for the production of food yeast are: the flowers of *Bassia* spp., [Mande *et al.* (150)]; the pentoses resulting from the acid hydrolysis of rice, straw, sawdust, and rice-husks by *Mycotorula japonica* and *Bacillus amyloliquefaciens* [Horii (114)]; hydrolyzed corn cobs and molasses [Marcilla & Hidalgo (151)]; juices of pears, apples, cherries, maraschino cherry brine [Adams & Hungate (1)]; beet-molasses [McGinnis (152)]; pressed-wood-board wastes [Takata & Kono (220)]; hydrolysates from the residuum of olive-packing plants, seaweeds [Suzuki (218); Tomiyasu *et al.* (226)] roots of *Taraxacum kok-saghyz* [Rafols (181)]; distillers corn mashes after prior conversion with cultures of *A. niger* [Pan *et al.* (165)]. Lee pointed out that while a substantial amount of yeast from breweries is recovered for food yeast, a much greater part is wasted (132). Grosser & Bernhauer (96) reported a yield of 25 per cent crude protein from a vigorously aerated suspension of cellulose inoculated with rapidly growing cultures of various species of *Penicillium*. A carbon balance study has been made by Whitaker (232) using 40 species from 21 genera of basidiomycetous wood-rotting fungi. Many of these fungi grew rapidly in aerated shake-flask culture. The microbial decomposition of cellulose, in-

cluding that by wood-rotting fungi, has recently been reviewed by Siu & Reese (202).

Fermentation Technology

Recent technological research in the fermentation industries has been largely directed towards the selection, improvement, maintenance, and pre-fermentation treatment of the inoculum; the testing of additional cheap raw materials; the determination of optimal conditions of aeration and agitation of the medium; and determination of the probable relationships between production in the various equipment units of the laboratory, pilot plant, and commercial fermenter.

The need for careful selection of strains is emphasized by Keipper (124), Parker (166), Corran (49), and Garner *et al.* (84). Improvement of synthetic potentiality may be obtained by strain selection, adaptation, induced mutation, hybridization, diploidization, or possibly by establishment of a heterokaryotic condition. For treatments of some of these aspects of microbial change, the reader is directed to reviews elsewhere in this volume. The following papers are also relevant (the subject matter is indicated after the authors). Christensen, Stakman & Christensen (43): variation and composition of the species; Christensen & Daly (45): adaptation; Lindegren (139), Foster (79), Pomper (173), Lederberg & Tatum (130a), Catcheside (41), and Fowell (83): microbial genetics, mutation, hybridization; Pontecorvo (174), Roper (189), Garnjobst (85), Lindegren *et al.* (140): heterokaryosis; Pontecorvo & Sermonti (175): synthesis of heterozygous diploid strains. Christensen & Daly (45) and Pomper (173) refer to the practical prospects of economic development through strain improvements. Stakman *et al.* (211) have described the use of uranium nitrate in the culture media for the production of mutants of fungi. The growth rate of the mycelium of a mutant mushroom so induced was from 5 to 7 times that of the original strain.

Pinsky & Stokes (171) contrary to the earlier suggestion by Hegarty (106) that adaptation occurred principally during physiological youth, offer evidence that certain adaptations are favoured by ageing. The rate of adaptation steadily decreased during active growth but was restored as the culture entered the stationary phase. Drews *et al.* (61) show that the amylolytic activity of *A. niger* in initially unfavourable media composed of industrial wastes will approach that of an optimal medium if opportunity for adaptation is provided. Prolonged culturing of *S. cerevisiae* at high temperatures yielded a thermophilic strain with increased maltase activity [Loginova (147)]. Komarova (126) describes the progressive adaptation of yeast to high concentrations of molasses.

Methods for the preservation of fungus cultures with a view to maintaining specific desirable characters are discussed by Fennell *et al.* (69). Lyophilization is usually the best method, followed by preservation in dried soil or under sterile oil.

General difficulties to be expected in submerged culture production methods are described by Perlman (168) and Wegrich & Shurter (230) using citric acid fermentation and penicillin production, respectively, as models.

A highly instructive series of studies aimed at predicting a relationship between laboratory scale fermentation and the commercial process have been made by Bartholemew and co-workers (14, 15, 16). A later paper in the series [Karow *et al.* (123)] provides mathematical treatment of the practical factors influencing oxygen transfer and agitation in submerged fermentations. Air pressures, flow rates, agitation, the numbers of spargers required, and shaft-horse-power per gallon needed for each impeller are considered. An earlier method for estimating efficiency of aeration and agitation is given by Cooper *et al.* (48). Hixson & Gaden (110) have developed "oxygen transfer equations" which allow comparative evaluation of different aeration systems, and Singh *et al.* (201) have discussed the influence of strain selection and of aeration and agitation on yield, and protein and vitamin content of several food yeasts. Olson & Johnson (163) describe laboratory culture experiments which suggest that it is possible to provide such efficient aeration as to decrease yields. Methods and equipment for sterilizing large quantities of air are discussed with regard to relative effectiveness and cost by Stark & Pohler (212).

An assembly for delivering a continuous supply of nutrients to series of shake-flasks for laboratory tests is described by Dale *et al.* (51). Analysis of growth curve data is said to offer a basis for predicting the cycle times of continuous fermentations [Adams & Hungate (1)]. Shu (200) describes an improved shake-flask assembly for exacting laboratory study of oxygen-uptake.

The influence of temperature on the growth and fermentation of yeast is considered mathematically by White & Munns (235). The influence of continuously poised pH values on the dissimilation of glucose by yeasts and by bacterial species is described by Neish & Blackwood (158). Yields of specific end-products may be more sensitive to pH than is growth of the organism.

General Comment

The foregoing review shows that it is now possible to produce valuable foods through microbial synthesis. However, it is problematic whether these possibilities will be exploited to any large degree. One handicap is that countries with the necessary reserve of technology and of plant capacity are not at present those which have the greatest need for additional food. Marketing and distribution problems may impose restriction, which could be further augmented by a general reluctance to eat new foods to which the palate is not yet educated. Cost estimates are discouraging. Peterson *et al.* (169) make a statement that reflects opinions of many others: "it is . . . doubtful if yeast protein can be produced at a price in the same range as low-cost protein—for example soybean protein. . . ." With regard to micro-

bial fat Wastler *et al.* (229) assert that "no microbial process can compete economically with edible fats derived from plants and animals." Dankwerts & Sellers (54) make similar comments, while Brasch (29) states of yeast fat, "because of its composition it cannot be conveniently refined and therefore would not compete favourably with vegetable oils for bulk industrial use." On the other hand, encouragement is provided by the penicillin industry which in its infancy produced penicillin at a cost of $7.50 per 100,000 units while current costs are about 1.8 cents per 100,000 units [Wegrich & Shurter (230)].

Taylor (223) comments that markets are not likely to absorb new protein foods at present costs, when the tried proteins of herring are available at the fish-docks at 1½ cents per lb., but still can not be effectively distributed. It should be pointed out, too, that additional toxicity trials seem to be indicated before foods from new species and after receiving different processing treatments should be offered for wide use. Liver damage resulting from specific diets containing microbial protein has already been discussed. The heat injury to dietary protein is the subject of a patent issued to Cannon *et al.* (37) while Crampton and co-workers (50) have shown that heat processing of vegetable oils may render them "toxic" by forming "cyclized" polymers of monomeric esters that produce measurable harmful effects when fed at the 2.5 per cent dietary level or higher.

Prospective future development may lie in the use of cheaper substrates, for which the forest and the ocean represent the chief massive reserves. The wood-rotting fungi may be a potentiality for the wider use of cheap cellulose and lignin, while Whitaker's (233) isolation of a single enzyme that converts cellulose to glucose, though at present far removed from commercial usefulness, may nevertheless point to a new approach for fermentation of cellulose. The use of rapidly growing thermophilic strains, symbiotic culture mixtures, other microorganisms (perhaps from the microflora of the rumen) that may produce high yields of the "deficient" amino acids, or fungi whose normal habitat is aquatic, may all merit further study. Several of the aquatic Phycomycetes are cultivatable upon common laboratory media [Machlis (149); Emerson (65)] but seem never to have been used for food synthesis even though adaptation of aerophilic species to submerged culture was one of the major early problems of the industry.

On the other hand, except as a means of reducing problems of waste disposal, the organisms now employed as sources of food may in time give way to the use of the photosynthetic green algae [Burlew (34)], to the nitrogen-fixing bacteria [Lee & Burris (131)], or perhaps to the photosynthetic and nitrogen-fixing blue-green algae [Fogg (77)].

Whatever the economic prospects it is fitting to be reminded of a recent statement by Dean (56) who said: "We know there are millions of undernourished children. We believe they could be better nourished if we used our plant resources more perfectly. We must find means of translating our beliefs into facts." With regard to this question one might well reflect upon

the theme from the new book by Boyd-Orr & Lubbock, that where there is hunger there can be no peace (27). Microbiology has shown one way to contribute. Is the world social structure elastic enough to accommodate to the stresses of implementing it?

LITERATURE CITED

1. Adams, S. L., and Hungate, R. E., *Ind. Eng. Chem.*, **42,** 1815–18 (1950)
2. Adriano, F. J., *Phillipine J. Agr.*, **4,** 1–9 (1933)
3. Agarwal, P. N., and Peterson, W. H., *Arch. Biochem.*, **20,** 59–74 (1949)
4. Almquist, H. J., *Ann. Rev. Biochem.*, **20,** 305–42 (1951)
5. Almquist, H. J., *Arch. Biochem. and Biophys.*, **46,** 250–51 (1953)
6. Aristovskaya, T. V., Litvinova, E. V., and Staszko, P., *Przemysl Chem.*, **5,** 552–57 (1949)
7. Arnaki, M., and Stary, Z., *Biochem. Z.*, **323,** 376–81 (1952)
8. Anderson, G. W., Slinger, S. J., and Pepper, W. F., *J. Nutrition*, **50,** 35–46 (1953)
9. Anderson, G. W., Slinger, S. J., Pepper, W. F., and Hauser, M. M., *J. Nutrition*, **50,** 47–58 (1953)
10. Arrazola, J. M., *4th Intern. Congr. Microbiol., Rept. Proc.*, 555 (Copenhagen, Denmark, July, 1947)
11. Arrazola, J. M., Marino, F. E., and Cia, R. A., *Bol. inst. nacl. invest. agron. (Madrid)*, **16,** 1–99 (1947)
12. Asano, M., Ukita, C., Tozawa, T., Korami, T., and Igeta, H., *J. Pharm. Soc. Japan*, **68,** 144–47 (1948)
13. Bass, A., and Hospodka, J., *Chem. Listy*, **46,** 243–45 (1952)
14. Bartholemew, W. H., Karow, E. O., Sfat, M. R., and Wilhelm, R. H., *Ind. Eng. Chem.*, **42,** 1801–9 (1950)
15. Bartholemew, W. H., Karow, E. O., Sfat, M. R., and Wilhelm, R. H., *Ind. Eng. Chem.*, **42,** 1810–15 (1950)
16. Bartholemew, W. H., Karrow, E. O., and Sfat, M. R., *Ind. Eng. Chem.*, **42,** 1827–30 (1950),
17. Barton-Wright, E. C., *J. Inst. Brewing*, **57,** 415–26 (1951)
18. Barton-Wright, E. C., *Wallerstein Lab. Communs.*, **15,** 115–29 (1952)
19. Bergner, K. G., *Deut. Lebensm, Rundshau*, **43,** 2–19 (1947)
20. Bernhard, K., *Cold Spring Harbor Symposia Quant. Biol.*, **13,** 26–28 (1948)
21. Bernhard, K., and Albrecht, H., *Helv. Chim. Acta*, **31,** 2214–20 (1948)
22. Bernhauer, K., and Rauch, J., *Biochem. Z.*, **319,** 77–93 (1948)
23. Bernhauer, K., Neithammer, A., and Rauch, J., *Biochem. Z.*, **319,** 94–101 (1948)
24. Bernhauer, K., and Rauch, J., *Biochem. Z.*, **319,** 102–19 (1948)
25. Bernhauer, K., Müller, P., and Theile, E., *Biochem. Z.*, **320,** 294–97 (1950)
26. Bernhauer, K., and Rauch, J., *Biochem. Z.*, **320,** 352–54 (1950)
27. Boyd-Orr, J., and Lubbock, J., *The White Mans Dilemma. Food and the Future*, (George Allen & Unwin, Ltd., London, England, 124 pp., 1953)
28. Brahmer, H., *Kgl. Lantbruksakad. Tidsks.*, **81,** 301–7 (1942)
29. Brasch, J. F., *J. Am. Oil Chemists' Soc.*, **28,** 396–99 (1951)
30. Braude, R., *J. Inst. Brewing*, **39,** 206–12 (1942)
31. Braude, R., Kon, S. K., and Porter, J. W. G., *Nutrition Abstr. & Revs.*, **23,** 472–96 (1953)
32. Brunner, R., *Mitt. Versuchs. Garungsgew., Wien.*, **3,** 101–3 (1949)

33. Bunker, H. J., *Chemistry & Industry*, 179–80 (1948)
34. Burlew, J. S., *Algal Culture from Laboratory to Pilot Plant* (*Carnegie Inst. Wash. Publ. No. 600*, 357 pp., 1953)
35. Campbell, L., *Pulp & Paper Mag. Can.*, **42**, 13–19 (1941)
36. Cannon, P. R., *Proc. Symposium on "Yeasts and Feeding,"* (Milwaukee, Wis., November, 1948)
37. Cannon, P. R., Jones, D. B., Lewis, H. B., Rose, W. C., and Stare, F. J., *Bull. Natl. Research Council; Reprint and Circ. Ser.*, No. 131 (1950)
38. Cannon, P. R., *Metabolism*, **1**, 49–57 (1952)
39. Carpenter, K. J., *Brit. J. Nutrition*, **5**, 243–49 (1951)
40. Carter, H. E., and Phillips, G. E., *Federation Proc.*, **3**, 123–28 (1944)
41. Catcheside, D. G., *The Genetics of Microorganisms* (Sir Isaac Pitman & Sons, Ltd., London, England, 223 pp., 1951)
42. Chiao, J. S., and Peterson, W. H., *J. Rood Chem.*, **1**, 1005–8 (1953)
43. Christensen, C. M., Stakman, E. C., and Christensen, J. J., *Ann. Rev. Microbiol.*, **1**, 61–84 (1947)
44. Christensen, C. M., *The Molds and Man* (University of Minnesota Press, Minneapolis, Minn., 244 pp., 1951)
45. Christensen, J. J., and Daly, J. M., *Ann. Rev. Microbiol.*, **5**, 57–70 (1951)
46. Clutterbuck, P. W., Lovell, R., and Raistrick, H., *Biochem. J.* (*London*), **26**, 1907–18 (1932)
47. Coates, M. E., *Brit. J. Nutrition*, **6**, 335–39 (1952)
48. Cooper, C. M., Fernstrom, G. A., and Miller, S. A., *Ind. Eng. Chem.*, **36**, 504–7 (1944)
49. Corran, H. S., *Chemistry & Industry*, 86–88 (1950)
50. Crampton, E. W., Common, R. H., Farmer, F. H., Wells, A. F., and Crawford, D., *J. Nutrition*, **49**, 333–45 (1953)
51. Dale, R. F., Amsz, J., Shu, P., Peppler, H. J., and Rudert, F. J., *Rev. Appl. Microbiol.*, **1**, 68–69 (1953)
52. Damm, H., *U. S. Pat. 2,340,011* (1944)
53. Dankwerts, P. W., and Sellers, E. S., *Food*, **21**, 459–61 (1952)
54. Dankwerts, P. W., and Sellers, E. S., *Food*, **22**, 23–25 (1935)
55. Darken, M. A., *Botan. Rev.*, **19**, 88–130 (1953),
56. Dean, R. F. A., *Brit. J. Nutrition*, **5**, 269–274 (1951)
57. Demmler, G., *Milchwissenschaft*, **5**, 11–17 (1950)
58. Diemair, W., and Neu, H., *Biochem. Z.*, **319**, 257–62 (1949)
59. Diemair, W., and Boresch, C., *Z. Lebensm. Untersuch. u. Forsch.*, **90**, 14–26 (1950)
60. Dirr, K., and Ruppert, A., *Biochem. Z.*, **319**, 163–73 (1949)
61. Drews, B., Specht, H., and Rothenbach, E., *Biochem. Z.*, **322**, 380–87 (1952)
62. Dunn, C. G., *Wallerstein Lab. Communs*, **15**, 61–78 (1952)
63. Eguchi, S., *J. Biochem* (*Japan*), **38**, 85–89 (1951)
64. Ehrensvard, G., *Cold Spring Harbor Symposia Quant. Biol.*, **13**, 81–87 (1948)
65. Emerson, R., *Ann. Rev. Microbiol.*, **4**, 169–200 (1950)
66. Enebo, L., Anderson, L. G., and Lundin, H., *Arch. Biochem.*, **11**, 383–395 (1946)
67. Fabel, K., *Milchwissenschaft*, **4**, 203–6 (1949)
68. Fabel, K., and Henssler, W., *Milchwissenschaft*, **5**, 192–97 (1950)
69. Fennell, D. I., Raper, K. B., and Flickinger, M. H., *Mycologia.*, **42**, 135–42 (1950)
70. Fink, H., Lechner, R., and Heinrich, E., *Biochem. Z.*, **183**, 71–91 (1936)
71. Fink, H., and Lechner, R., *Biochem. Z.*, **286**, 83–98 (1936)

72. Fink, H., Haeseler, G., and Schmidt, M., *Z. Spiritusind.*, **60**, 74–82 (1937)
73. Fink, H., Haehn, H., and Hoerburger, W., *Chemicker Ztg.*, **61**, 689–744 (1937)
74. Fink, H., Lechner, R., and Krebs, J., *Biochem. Z.*, **301**, 143–49 (1939)
75. Fiore, J. V., *Arch. Biochem.*, **16**, 161–68 (1948)
76. Flodin, N. W., *J. Agr. Food. Chem*, **1**, 222–35 (1953)
77. Fogg, G. E., *Endeavour*, **6**, 172–75 (1947)
78. Foster, J. W., *Chemical Activities of Fungi* (Academic Press, Inc., New York, N. Y., 648 pp., 1951)
79. Foster, J. W., *Ann. Rev. Microbiol.*, **5**, 101–20 (1951)
80. Fruton, J. S., and Simmonds. S., *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 55–63 (1949)
81. Fruton, J. F., *Wallerstein Lab. Communs*, **15**, 133–46 (1952)
82. Fowden, L., *Biochem. J.* (*London*), **50**, 355–58 (1952)
83. Fowell, R. R., *J. Inst. Brewing*, **57**, 180–95 (1951)
84. Garner, H. R., Koffler, H., Tetrault, P. A., Fahmy, M., Mallett, V. M., Faust, R. A., Phillips, R. L., and Bohonos, N., *Am. J. Botany*, **40**, 289–96 (1953)
85. Garnjobst, L., *Am. J. Botany*, **40**, 607–14 (1953)
86. Goodwin, T. W., *Biochem. J.* (*London*), **48**, 154–63 (1951)
87. Goodwin, T. W., *The Comparative Biochemistry of the Carotenoids* (Chapman & Hall, Ltd., London, England, 356 pp., 1952)
88. Goodwin, T. W., *Botan. Rev.*, **18**, 291–316 (1952)
89. Goodwin, T. W., and Lijinsky, W., *Biochem. J.* (*London*), **50**, 268–73 (1952)
90. Goodwin, T. W., and Griffiths, L. A., *Biochem. J.* (*London*), **52**, 499–501(1952)
91. Gorcica, H. J., Peterson, W. H., and Steenboch, H., *J. Nutrition*, **9**, 691–700 (1935)
92. Gorcica, H. J., Peterson, W. H., and Steenboch, H., *J. Nutrition*, **9**, 701–14 (1935)
93. Graham, V. E., Gibson, D. L., Klemmer, H. W., and Naylor, J. M., *Can. J. Technol.*, **31**, 85–91 (1953)
94. Graham, V. E., Gibson, D. L., Klemmer, H. W., *Can. J. Technol.*, **31**, 92–97 (1953)
95. Graham, V. E., Gibson, D. L., and Lawton, W. C., *Can. J. Technol.*, **31**, 109–12 (1953)
96. Grosser, A., and Bernhauer, K., *Biochem. Z.*, **320**, 162–73 (1950)
97. György, P., and Goldblatt, H., *J. Exptl. Med.*, **89**, 245–68 (1949)
98. György, P., Rose, C. S., Tomarelli, R. M., and Goldblatt, H., *J. Nutrition*, **41**, 265–71 (1950),
99. Hall, H. H., Benedict, R. G., Wiesen, C. F., Smith, C. E., and Jackson, R. W., *Appl. Microbiol.*, **1**, 124–29 (1953)
100. Harris, E. E., Beglinger, E., Hajny, G. J., and Sherrard, E. C., *Ind. Eng. Chem.*, **37**, 12–23 (1945)
101. Harris, E. E., and Johnson, M. J., *Paper Ind. and Paper World*, **30**, 940–43 (1948)
102. Harris, E. E., Hannan, M. L., Marquardt, R. R., and Bubl. J. L., *Ind. Eng. Chem.*, **40**, 1216–20 (1948)
103. Harris, E. E., Hannan, M. L., and Marquardt. R. R., *Ind. Eng. Chem.*, **40**, 2068–77 (1948)
104. Harris, E. E., Hajny, G. J., and Johnson, M. C., *Ind. Eng. Chem.*, **43**, 1593–96 (1951)

105. Heald, P. J., *Biochem. J.* (*London*), **50,** 503–8 (1952)
106. Hegarty, C. P., *J. Bact.*, **37,** 145–54 (1939)
107. Heines, V., and Nord, F. F., *Arch. Biochem.*, **11,** 521–30 (1946)
108. Hilditch, T. P., and Shrivastava, R. K., *Biochim. et Biophys. Acta.*, **22,** 80–85 (1948)
109. Himsworth, H. P., and Lindan, O., *Nature*, **163,** 30 (1949)
110. Hixson, A. W., and Gaden, E. L., *Ind. Eng. Chem.*, **42,** 1792–1801 (1950)
111. Hock, A., and Fink, H., *Hoppe-Seyler's Z. physiol. Chem.*, **278,** 136–43 (1943)
112. Hoover, S. R., *Science*, **114,** 213 (1951)
113. Hoover, S. R., *Sewage and Ind. Wastes*, **25,** 201–9 (1953)
114. Horii, K., *J. Fermentation Assoc.* (*Japan*), **7,** 278–80 (1949)
115. Humfeld, H., *Science*, **107,** 73 (1948)
116. Humfeld, H., and Sugihara, T. F., *Mycologia*, **44,** 605–20 (1952)
117. Inskeep, G. C., Wiley, A. J., Holderby, J. M., and Hughes, L. P., *Ind. Eng. Chem.*, **43,** 1702–11 (1951)
118. Iwata, Y., *J. Fermentation Assoc.* (*Japan*), **8,** 49–53 (1950)
119. Jacobs, M. B., *The Chemistry and Technology of Food and Food Products*, 2nd ed., **II,** 1050–52 (Interscience Publishers, Inc., New York, N. Y., 934 pp., 1951)
120. Johnson, M. J., *Ann. Rev. Microbiol.* **1,** 159–72 (1947)
121. Jukes, T. H., and Williams, W. L., *Pharmacol. Revs.*, **5,** 381–420 (1953)
122. Kapfhammer, J., *Ernährung*, **9,** 29–32 (1944)
123. Karow, E. O., Bartholemew, W. H., and Sfat, M. R., *J. Agr. Food Chem.*, **1,** 302–6 (1953),
124. Keipper, C. H., *Food Inds.*, **22,** 1375–80 (1950)
125. Kleinzeller, A., *Advances in Enzymol.*, **8,** 299–341 (1948)
126. Komarova, L. I., *Trudy Inst. Mikrobiol., Akad. Nauk, S.S.S.R.*, **1,** 136–41 (1951)
127. Kreipe, H., *Arch. Tierernähr.*, **1,** 56–64 (1950)
128. Kunze, R., *Mitt. Versuchs. Garungagew., Wien.*, **2,** 44–51 (1950); abstracted in *J. Inst. Brewing*, **56,** 335 (1950)
129. Kurth, E. F., *Ind. Eng. Chem.*, **38,** 204–7 (1946)
130. Laufer, L., Schwartz, D. R., and Stewart, E. D., *J. Agr. Food Chem.*, **1,** 91–95 (1953)
130a. Lederberg, J., and Tatum, E. L., *Science*, **118,** 169–74 (1953)
131. Lee, S. B., and Burris, R. H., *Ind. Eng. Chem.*, **35,** 354–57 (1943)
132. Lee, S. B., *Ind. Eng. Chem.*, **41,** 1869–79 (1949)
133. Levine, H., Oyaas, J. E., Wasserman, L., Hoogerbeide, J. C., and Stern, R. M., *Ind. Eng. Chem.*, **41,** 1665–68 (1949)
134. Leviton, A., and Whittier, E. O., *J. Dairy Sci.*, **33,** 402 (1950)
135. Lindan, O., and Work, E., *Biochem. J.* (*London*), **48,** 337–44 (1951)
136. Lindan, O., and Work, E., *Biochem. J.* (*London*), **48,** 344–49 (1951)
137. Lindan, O., and Work, E., *Biochem. J.* (*London*), **55,** 554–61 (1953)
138. Lindan, O., and Work, E., *Biochem. J.* (*London*), **55,** 562–65 (1953)
139. Lindegren, C. C., *Ann. Rev. Microbiol.*, **2,** 47–70 (1948)
140. Lindegren, C. C., Braham, J. E., and De Dios Calle, J., *Nature*, **172,** 800–2 (1953)
141. Lintzel, W., *Chem. Ztg.*, **67,** 33–38 (1943)
142. Lindquist, W., *Biochim. et Biophys. Acta.*, **10,** 443–48 (1953)
143. Lindquist, W., *Biochim. et Biophys. Acta.*, **10,** 580–87 (1953)
144. Lindquist, W., *Biochim. et Biophys. Acta.*, **11,** 90–94 (1953)

146. Loesecke, H. W. von, *J. Am. Dietet, Assoc.*, **22**, 485–94 (1946)
147. Loginova, L. G., *Mikrobiologiya*, **21**, 684–89 (1952); *Chem. Abstracts*, **47**, 6600 (1953)
148. Lundin, H., *J. Inst. Brewing*, **56**, 17–28 (1950)
149. Machlis, L., *Am. J. Botany*, **40**, 189–95 (1953); *Am. J. Botany*, **40**, 450–59 (1953); *Am. J. Botany*, **40**, 460–64 (1953)
150. Mande, B. A., Andreasen, A. A., Sreenivasaya, M., and Kolachov, P., *Ind. Eng. Chem.*, **41**, 1451–54 (1949)
151. Marcilla, J., and Hidalgo, L., *Microbiol. Española*, **3**, 125–30 (1950)
152. McGinnis, R. A., *Beet-sugar Technology*, 114 (Reinhold Publishing Corp., New York, N. Y., 574 pp., 1951)
153. McLean, J. R., and Beveridge, J. M. R., *J. Nutrition*, **47**, 41–55 (1952)
154. McNaught, M. L., *Biochem. J. (London)*, **49**, 325–31 (1951)
155. Mondolfo, H., and Hounie, E., *Rev. assoc. bioquim. argentina*, **15**, 416–18 (1951)
156. Morita, E., *J. Chem. Soc. Japan, Pure Chem. Sect.*, **73**, 909–12 (1952)
157. Muller, W. R., *Milchwissenchaft*, **4**, 147–53 (1949)
158. Neish, A. C., and Blackwood, A. C., *Can. J. Technol.*, **39**, 123–31 (1951)
159. Neuberg, C., *Am. Brewer*, **68**, 35–40 (1950)
160. Nielsen, N., Sandegren, E., and Ljungdahl, L., *Nature*, **164**, 1055 (1949)
161. Nilsson, R., Enebo, L., Lundin, H., and Myrback, K., *Svensk kem. Tidskr.*, **5**, 196 (1945)
162. Nord, F. F., and Mull, R. P., *Advances in Enzymol.*, **5**, 165 (1945)
163. Olson, B. H., and Johnson, M. J., *J. Bact.*, **57**, 235–40 (1949)
164. Pan. S. C., Andreasen, A. A., and Kolachov, P., *Arch. Biochem.*, **23**, 419–33 (1949)
165. Pan, S. C., Andreasen, A. A., and Kolachov, P., *Arch. Biochem.*, **30**, 6–13 (1951)
166. Parker, A., *Brewers' Guild J.*, **37**, 1–22 (1951)
167. Pavcek, P. L., *Wallerstein Lab. Communs.*, **13**, 369–78 (1950)
168. Perlman, D., *Econ. Botany*, **3**, 360–74 (1949)
169. Peterson, W. H. Snell, J. F., and Frazier, W. C., *Ind. Eng. Chem.*, **37**, 30–35 (1945)
170. Pfeifer, V. F., Tanner, F. W., Vojnovich, C., and Traufler, D. H., *Ind. Eng. Chem.*, **42**, 1776–81 (1950)
171. Pinsky, M. J., and Stokes, J. L., *J. Bact.*, **64**, 337–45 (1952)
172. Piskur, M. J., *J. Am. Oil Chemists' Soc.*, **29**, 136–48, 180–97 (1952)
173. Pomper, S., *Wallerstein Lab. Communs.*, **14**, 31–42 (1951)
174. Pontecorvo, G., *Cold Spring Harbor Symp. Quant. Biol.*, **11**, 193–201 (1946)
175. Pontecorvo, G., and Sermonti, G., *Nature*, **172**, 126–27 (1953)
176. Porges, N., Pepinsky, J. B., and Jasewicz, L., *J. Dairy Sci.*, **34**, 615–21 (1951)
177. Porter, J. R., *Bacterial Chemistry and Physiology* (John Wiley & Sons, Inc., New York, N. Y., 1073 pp., 1948)
178. Pridham, T. G., *Econ. Botany*, **6**, 187–205 (1952)
179. Preuss, L. M., Peterson, W. H., Steenboch, H., and Fred, E. B., *J. Biol. Chem.*, **90**, 369–84 (1931)
180. Preuss, L. M., Gorcica, H. J., Greene, H. C., and Peterson, W. H., *Biochem. Z.*, **246**, 401–13 (1932)
181. Rafols, W. de, *Bol. inst. nacl. invest. agron. (Madrid)*, **12**, 57–103 (1952)
182. Reed, G., *Food Tech.*, **6**, 339–41 (1952)
183. Reinartz, F., and Lafos, H., *Angew Chem.*, **62**, 572–75 (1950)
184. Reinhardt, W. O., *Nature*, **172**, 915–16 (1953)

185. Rippel, A., *Naturwissenschaften*, **31**, 248–57 (1943)
186. Roberts, L. P., *Nature*, **165**, 494–95 (1950)
187. Roberts, L. P., *Nature*, **172**, 351–52 (1953)
188. Robinson, R. F., *Science Monthly*, **6**, 149–54 (1952)
189. Roper, J. A., *Experientia*, **8**, 14 (1952)
190. Rose, W. C., *Science*, **86**, 298 (1937)
191. Saeman, J. F., Locke, E. G., Dickerman, G. K., *FIAT Final Report*, 499 (1945)
192. Sahyun, M., *Proteins and Amino Acids in Nutrition* (Reinhold Publishing Corp., New York, N. Y., 566 pp., 1948)
193. Sandegren, E., Ekstrom, D., Nielsen, N., *Acta Chem. Scand.*, **4**, 1311–13 (1951)
194. Savard, K., and Grant, G. A., *Science*, **104**, 459–60 (1946)
195. Schendel, H. E., and Johnson, B. C., *J. Agr. Food Chem.*, **2**, 23–24 (1954)
196. Schormüller, J., *Z. Lebensm. Untersuch. u. Forsch.*, **89**, 481–505 (1949)
197. Schwarz, K., *Proc. Soc. Exptl. Biol. Med.*, **80**, 319–23 (1952)
198. Seeley, R. D., Zeigler, H. F., and Sumner, R. J., *Cereal Chem.*, **27**, 50–60 (1950)
199. Shaefer, A. E., Salmon, W. D., and Strength, D. R., *Proc. Soc. Exptl. Biol. Med.*, **71**, 193–99 (1949)
200. Shu, P., *J. Agr. Food Chem.*, **1**, 1119–23 (1953)
201. Singh, K., Agarwal, P. N., and Peterson, W. H., *Arch. Biochem.*, **18**, 181–93 (1948)
202. Siu, R. G. H., and Reese, E. T., *Botan. Rev.*, **19**, 377–416 (1953)
203. Skinner, J. T., Peterson, W. H., and Steenboch, H., *Biochem. Z.*, **267**, 169–78 (1933)
204. Skinner, C. S., *J. Bact.*, **28**, 95–106 (1934)
205. Skinner, C. E., and Muller, A. E., *J. Nutrition*, **19**, 333–44 (1940)
206. Smith, E. L., *Nutrition Abstr. & Revs.*, **20**, 796–809 (1951)
207. Smithies, W. R., *Biochem. J. (London)*, **51**, 259–64 (1952)
208. Smythe, C. V., *Econ. Botany*, **5**, 126–44 (1951)
209. Sperber, E., *Arkiv. Kemi Mineral Geol.*, **21A**, 1–136 (1945)
210. Springer, R., *Z. Lebensm. Untersuch. u. Forsch.*, **89**, 353–62 (1949)
211. Stakman, E. C., Daly, J. M., Gattani, M. L., and Wahl, I., *Science*, **108**, 554–55 (1948)
212. Stark, W. H., and Pohler, G. M., *Ind. Eng. Chem.*, **42**, 1789–92 (1950)
213. Starkey, L. R., *J. Bact.*, **51**, 33–38 (1946)
214. Suminoe, K., and Ketsu, B., *J. Fermentation Assoc. (Japan)*, **8**, 189–90 (1950); *Chem. Abstracts*, **46**, 11500 (1952)
215. Sure, B., *J. Am. Dietet. Assoc.*, **22**, 494, 766 (1946)
216. Sure, B., *J. Nutrition*, **36**, 59–65 (1948)
217. Sure, B., and House F., *Arch. Biochem.*, **20**, 55–58 (1949)
218. Suzuki, N., *Bull. Fac. Fisheries, Hokkaido Univ.*, **3**, 118–20 (1952)
219. Takata, R. J., *J. Soc. Chem. Ind.*, Japan, **32**, 243–44 (1929)
220. Takata, O., and Kono, A., *Chem. Abstracts*, **47**, 4547 (1952)
221. Tanner, F. W., Wickerham, L. J., and Van Lanen, J. M., *U. S. Pat. 2,445,128* (1948)
222. Tauber, H., *The Chemistry and Technology of Enzymes* (John Wiley & Sons, Inc., New York, N. Y., 550 pp., 1948)
223. Taylor, H. F., *Chem. Eng. News*, **32**, 134 (1954)
224. Thaysen, A. C., and Morris, M., *Nature*, **154**, 526 (1943)
225. Thaysen, M., *A Venture in Practical Nutrition* (Colonial Food Yeast, Ltd., London, England, 1944)

226. Tomiyasu, Y., and Zenitani, B., *J. Agr. Chem. Soc.* Japan, **25,** 406–10 (1952)
227. Vinson, L. J., *Frontier, The,* 9–12, 24–25 (1945)
228. Vinson, L. J., Cerecedo, L. R., Mull, R. P., and Nord, F. F., *Science,* **101,** 388–89 (1945)
229. Wastler, T. A., Daugherty, P. M., and Sineath, H. H., *Georgia Inst. Technol., Bull. No. 13* (1951)
230. Wegrich, O. G., and Shurter, R. A., *Ind. Eng. Chem.,* **45,** 1153–60 (1953)
231. Weinhouse, S., Millington, R. H., and Strassman, M., *J. Am. Chem. Soc.,* **73,** 1421–23 (1951)
232. Whitaker, D. R., *Can. J. Botany,* **29,** 159–75 (1951)
233. Whitaker, D. R., *Arch. Biochem. and Biophys.,* **43,** 253–68 (1953)
234. White, J., and Munns, D. J., *J. Inst. Brewing,* **56,** 261–65 (1950)
235. White, J., and Munns, D. J., *J. Inst. Brewing,* **57,** 280–84 (1951)
236. Wiley, A. J., Dubey, G. A., Lueck, B. F., and Hughes, L. P., *Ind. Eng. Chem.,* **42,** 1830–33 (1950)
237. Woodbine, M., Gregory, M. E., and Walker, T. K., *J. Exptl. Botany,* **2,** 204–11 (1951)
238. Woolley, D. W., Berger, J., Peterson, W. H., and Steenboch, H., *J. Nutrition,* **16,** 465–76 (1938)
239. Yokoyama, Y., *J. Antibiotics (Japan),* **4,** 95–102 (1951)

CYTOPATHOLOGY OF VIRUS INFECTIONS

(Particular Reference to Tissue Culture Studies)

By John F. Enders

Research Division of Infectious Diseases,
The Children's Medical Center
and
Department of Bacteriology and Immunology,
Harvard Medical School,
Boston, Massachusetts

INTRODUCTION

Primarily this review aims to summarize the principal observations on the effect of certain viruses on cells cultivated *in vitro*.[1] The viruses to be considered will be limited to those pathogenic for warm-blooded animals. Excluded, therefore, are those attacking bacteria, plants, insects, and other poikilothermic organisms. A further limitation will consist in the omission, with a few exceptions, of the effect of viruses on malignant cells, since the oncolytic properties of viruses will be reviewed elsewhere in this volume (3).

The significance of the abnormal changes induced by viruses in cultured cells both from the biologic and practical points of view has in the past not been widely recognized, although many who studied viral multiplication in such systems examined their material for the presence of inclusion bodies or other manifestations of injury. The recent demonstration (4) that the agents of poliomyelitis can be propagated *in vitro* in extraneural tissues with the accompaniment of degenerative changes appears to have stimulated renewed interest in cytopathic phenomena induced by viruses under these conditions. Of late publications on this subject have greatly increased. But we are as yet far from possessing sufficient data for a complete account of the changes induced by even the mammalian viruses that can be cultivated *in vitro*. Indeed much investigation still lies ahead which, when completed, should add to our understanding of the basic interactions between host and virus as well as provide practical and simple means for the recognition and possibly the control of viral diseases. The second objective, therefore, of this survey is to indicate directly or indirectly areas where knowledge is still incomplete or lacking.

GENERAL REMARKS CONCERNING THE CYTOPATHOGENICITY OF VIRUSES IN TISSUE CULTURE

By "viral cytopathogenicity" is understood the capacity to induce any

[1] Recent reviews of the literature on the cultivation of viruses in tissue culture have been published by Sanders, Kiem & Lagunoff (1) and Robbins & Enders (2), where references to earlier summaries of this sort will be found.

demonstrable departure from the normal either in the morphological or functional properties of cells. Cellular changes are referred to as "cytopathogenic effects" of the virus or "cytopathic" changes. This broad interpretation of "cytopathogenicity" contrasts with the restricted sense in which it has been applied in a recent review by Lynn & Morgan (5) to include only that activity of a virus resulting in visible cellular changes "such as distortion and pyknosis of nucleus, granularity of cytoplasm, irregularity of cell shape, and cellular disintegration."

Most cytopathogenic effects in tissue cultures so far described consist of morphological disturbances. For convenience these may be classified in three groups, although two or more may occur successively in the same infected cell population: Group 1. Rounding or assumption of irregular or bizarre outlines, granulation of cytoplasm, swelling of the cell, nuclear swelling, pyknosis, fragmentation of nuclear chromatin, cell death, and disintegration; Group 2. Formation of inclusion bodies in either cytoplasm or nucleus; Group 3. Formation of giant cells or coalescence of cells into syncytial-like masses.

In certain instances cytopathogenicity may not be expressed by unmistakable morphological alterations but may be reflected in disorders of cellular function which at present can be convincingly demonstrated only by indirect means. It is not yet certain whether such effects eventually lead to cell death. At present, however, there are indications that cells may survive and continue to participate in viral synthesis of virus for prolonged periods. At present no functional changes in the absence of morphological abnormalities are known that can be with certainty attributed to viral activity. Data exist, however, suggesting that infection of cells with agents not appearing to produce morphologic changes may alter cellular metabolism [see, for example, Womack & Kass (6)]. Future investigation, therefore, of such systems may reveal "biochemical lesions" of theoretical and practical importance.

The phenomena mentioned above under Group 1 changes may be evoked by many noxious agents. Accordingly, they cannot alone be considered as necessarily the result of viral activity. To prove this certain control procedures (serial cultivation, prevention of changes by homologous antibody, etc.) must be applied. Familiarity, however, with the effects of a specific virus in a given cell system often enables the observer to conclude tentatively that this virus is responsible.

Of morphological indices of viral injury, the formation of inclusion bodies (Group 2 above) is the most characteristic, although again this process cannot be accepted as conclusive evidence of viral activity since certain chemical as well as other unknown factors may condition its development. Inclusion bodies were the first cytopathic changes to be sought for *in vitro* and employed as criteria of infection. As indices of viral multiplication, however, they are less useful than the changes of Group 1, because these structures can be unmistakably demonstrated only in stained preparations.

Certain of the cytopathic manifestations of Group 3 have only been

recently recognized. More study will be required before their full significance and the factors responsible for them are understood. Nevertheless, already they appear to afford useful criteria for identification of the responsible viruses.

Cytopathogenicity *in vitro* is influenced by factors some of which are known while many remain to be defined. At the outset a few of those now recognized will be mentioned as an introduction to the review of recorded observations on the behavior of individual agents. Of primary importance is the species from which the cells are derived. Analogous to the host range of a virus is its cytopathogenic range in cultivated cells. But correlation between susceptibility of the organism and its cells *in vivo* does not always exist. For although this correlation frequently obtains, the tissues of a susceptible species occasionally fail to support viral multiplication while the converse of this situation also occurs.

Cell type is, with certain viruses, a determining factor. Thus an agent may attack and destroy epithelial cells present in a culture leaving fibroblasts intact. Experiments with strains consisting of a single cell type have been few, but the results indicate that the cytotropic properties of viruses *in vivo* may be retained *in vitro*. Once more, however, there is no absolute relationship between cytotropism *in vivo* and *in vitro*.

The age of the donor of tissue may influence cytopathogenicity. Just as young animals are frequently more susceptible to infection so their tissues may be more vulnerable to injury by the virus, yet again this correlation is not invariable. Most of the pertinent data indicate that acquired immunity to viral infections is not reflected by an increased cellular resistance, a fact advantageous from the technical point of view since it eliminates concern over the immunologic status of the donor animal.

The intensity and degree of cytopathic injury may vary according to the strain of virus or the conditions under which it has been propagated prior to its study in tissue culture. The investigator should be prepared to encounter such variations in the study of a number of representatives of a viral species. Moderate or weak cytopathogenicity may sometimes be enhanced by serial passage *in vitro*.

Environmental factors in the culture may tend to enhance or suppress cytopathogenic activity. Of these many have not yet been defined, but there is evidence that composition of the medium, temperature of incubation, and period of cultivation of the cells before addition of virus may all be determinants.

MORPHOLOGIC MANIFESTATIONS OF VIRAL CYTOPATHOGENICITY

In summarizing reports on cytopathic changes induced *in vitro* we shall consider the responsible agents under three headings: (A) Viruses causing only cellular degeneration (changes of Group 1); (B) Viruses causing formation of inclusion bodies and cell degeneration (changes of Groups 1 and 2);

(C) Viruses causing formation of multinucleated cells or syncytial masses and degeneration with or without inclusion bodies and exhibiting a tendency to produce "foci" of infected cells (changes of Groups 1, 2, and 3).

Viruses Causing Only Cellular Degeneration

The sequence of changes induced by certain of these viruses have been described in detail; in other cases we have merely evidence that they caused death of cells.

Acute respiratory disease viruses.—In cultures of either human adult tracheal epithelium or HeLa cells[2] Hilleman & Werner (7) isolated five agents exhibiting similar cytopathogenic properties from cases of primary atypical pneumonia or epidemic acute respiratory illness. In HeLa cells signs of cellular degeneration observed 24 hr. after addition of virus terminated after 48 hr. in complete destruction. The cells lost their protoplasmic processes, rounded and eventually aggregated into masses consisting of dark, granular, and shrunken elements. At times the cell surface was covered with small globoid or vermiform extrusions. Stained preparations revealed essentially the same changes and in addition intense basophilia of nucleus and cytoplasm, nuclear degeneration, margination of chromatin, increased granularity, and pyknosis or reticular formation with apparent vacuolization. No inclusion bodies were seen, however. Antibodies capable of preventing these changes developed during the disease. Antigen fixing complement in the presence of convalescent phase sera was also demonstrated in the fluid of cultures showing cytopathic changes. Evidence was obtained of an antigenic relationship between these agents and the adenoid degenerative agent recently described by Rowe and his co-workers (9). Recognition of this new group of viruses through their cytopathogenic effects affords an excellent example of the peculiar value of the tissue culture method; since, so far, no susceptible animal has been found.

Coxsackie viruses.—The capacity of certain members of this group to multiply *in vitro* has been determined and correlated with cytopathogenicity for various tissues. While many strains can be propagated in this manner, those capable of causing recognizable cellular injury appear to be limited in number. The first direct observations on cytopathic changes were made by Riordan, Ledinko & Melnick (10) and Stulberg, Schapira & Eidam (11), although earlier one strain isolated in suspended cell cultures was indirectly recognized by Robbins *et al.* (12) as exerting a destructive effect on the basis of reduced acid production. Granulation, rounding and ultimate fragmentation of fibroblasts occur in cultures of monkey testicular tissue (10), mouse interscapular fat pad tissue (11), and human embryonic skin and muscle (13). In fibroblastic growth from original explants degeneration often begins in the adjacent cells and later extends radially through the outgrowth (10). Certain

[2] For an account of the origin of these cells and method of cultivation *in vitro* see reference 8.

strains cause degeneration of human renal epithelial cells [(13) and unpublished observations by Enders and co-workers]. The effect is not to be distinguished with certainty from that of poliomyelitis, although swelling and the development of "bizarre" forms is not usually as striking.

Marked differences in growth capacity exhibited by various strains are revealed in experiments of Weller and his associates (13). Employing human embryonic and mature tissues in roller tubes or suspended cell cultures and three strains (2 of Group A, 1 of Group B), they found that one multiplied only in cultures of embryonic brain tissue, another in embryonic brain and intestine and mature kidney but not in embryonic skin and muscle; but cytopathogenic effects were noted only in cultures of brain and intestinal tissues. A third affected cells of both embryonic and mature tissues. Failure to grow in culture, at least in the presence of a given type of tissue, appears to be characteristic of other strains. Duncan and his associates (14) isolated 19 viruses in suckling mice from cases of paralytic and nonparalytic poliomyelitis; 6 proliferated in suspended cell cultures of monkey testis. Stulberg and his co-workers (11) saw degenerative changes in cells from mouse skeletal muscle and interscapular fat pad tissues infected with a Group B strain. In contrast a representative of Group A failed to multiply under these conditions. Superficially these agents appear to behave similarly when inoculated into suckling mice since Godman *et al.* (15) found that the Group B strain was highly pathogenic for fatty tissues. However, since the Group A strain failed to grow *in vitro* in skeletal muscle a true parallelism in cytopathogenic properties was not demonstrated. Differences in cytopathogenicity for cultured cells may provide helpful criteria for the eventual classification of this confusing group.

Cytopathogenic "C" viruses have been recovered repeatedly in cultures in attempts to isolate agents of poliomyelitis (10, 12, 14). Slight differences in appearance of affected cells may aid in distinguishing them from the latter, but definite differential evidence consists in the failure of antipoliomyelitis sera to prevent cytopathic change.

Equine encephalomyelitis viruses.—Eastern and Western strains destroy cells rapidly in tissue culture. The Venezuelan agent multiplies *in vitro* (16) but its cytopathogenicity has not been investigated. Huang was the first to recognize clearly the practical significance of cellular degeneration caused by the Western strain. In 1942 he showed (17) that infected fragments of chick embryonic tissue in suspended cell cultures when later explanted in plasma clots failed to develop fibroblastic growth. He utilized this phenomenon in the assay of viral infectivity and specific neutralizing antibody. Comparative tests in cultures and mice showed the former to be a more sensitive indicator of virus (18). In infected cultures glycolysis, as compared with control cultures, was diminished (19). Realizing that this was an indirect manifestation of viral cytopathogenicity Huang employed changes in pH of the culture medium in the titration of infectivity. He (20) also demonstrated interference of the cytopathogenic effects of equine encephalitis virus by the noncytopa-

thogenic St. Louis virus when the latter was first introduced into the culture. By determining the smallest amount of St. Louis virus causing such interference he was able to assay its infectivity. Taylor (21) has recently reported that influenza A virus interferes with the cytopathogenic effect of Eastern and Western strains in cultures of chick chorioallantoic membrane. When the cytopathogenicity of poliomyelitis virus was demonstrated certain of Huang's procedures were adopted in the investigation of its behavior. As contributions to the methodology of viral propagation their value cannot be overemphasized.

Bang & Gey (22), in an analysis of the effects of the eastern strain in roller tube cultures of 13 established lines of rat cells, discovered wide variations in cell susceptibility. Among three strains of fibroblasts, one failed to support viral multiplication and exhibited no cytopathic changes. Another, derived from a different tissue of the same animal, was moderately susceptible. With this strain an apparent equilibrium was established between cell destruction and proliferation which resulted in continued production of virus over periods exceeding four months. Cells of a third strain were rapidly killed by the virus. Also of much interest was the finding that a strain of malignant sarcoma cells, developed from the virus-resistant strain of normal rat fibroblasts, proved completely susceptible to the virus. Likewise studied were two strains of human malignant cells; in one, the cells were completely destroyed by the virus while in the other a proportion proved resistant. These results afford an excellent example of how differences in the same type of cell derived from the same animal may condition cytopathogenicity.

Taking advantage of the capacity of these agents to destroy cells, Dulbecco & Vogt (23, 24) have devised a technique for quantitative estimation of infective particles analogous to the plaque counting method for bacteriophage. This development opens the way to more accurate analyses of problems relating to the multiplication of many mammalian viruses. The procedure consists essentially in the preparation of a suspension of individual cells obtained by treating certain tissues intensively with trypsin. With this suspension monolayer cultures of cells are established in suitable containers. After the addition of diluted virus, nutrient medium in agar is applied. When set, this overlying layer confines virus as it multiplies to the region of the cell which is first infected. Following incubation a "plaque" or circular zone develops, resulting from the destruction of adjacent cells to which the virus has spread from the one primarily infected, presumably by direct contact. Evidence has been adduced indicating that each plaque represents the ultimate effect of a single infective unit.

The wide range of cellular susceptibility to these agents has already been illustrated. In addition to the cells that have been just mentioned it has been lately shown that (25) monkey testicular cells are completely destroyed by the Western strain. The findings *in vitro*, then, are consistent with the fact that these viruses are pathogenic for many species of birds and mammals.

Foot and mouth disease virus.—Observations made by Striegler (26) and

Köbe & Fertig (27) indicate that susceptible cells are injured or killed by the virus. The former investigated its effect in what they considered to be pure cultures of guinea pig iris epithelium and guinea pig fibroblasts. Associated with viral multiplication in the epithelial cells were degenerative changes resulting in the loss of many; the remainder failed to proliferate when explanted. No growth of virus or degeneration occurred in cultures of fibroblasts. Striegler, using guinea pig testis in Maitland or Rivers cultures, noted that the infected fragments lost their cuboidal outlines and became yellow and fatty in appearance, suggesting the occurrence of massive necrosis. Controls were not mentioned, however. In consequence, the specificity of this phenomenon remains in doubt.

Restriction of viral multiplication to epithelial elements had previously been noticed by Hecke (28), who employed the foetal skin of guinea pigs. He found that multiplication was increased in tissues from 5 cm. embryos as compared with those from $2\frac{1}{2}$ cm. specimens. This difference he attributed to the development of less fibroblastic outgrowth from the older tissue. Frenkel (29), who in recent years has used cultures of bovine lingual epithelium for the preparation of vaccine, has described the formation of vesicles *in vitro* which resemble histologically those observed *in vivo*. It would seem desirable to undertake a reinvestigation of the cytopathogenic properties of these agents with the purpose of confirming the findings of earlier workers in regard to their limited cytotropism. Also of value might be a comparative study of the cytopathogenicity of the three antigenic types.

Newcastle disease virus.—Because of its marked pathogenicity for the living host NDV might be expected to produce a severely damaging effect *in vitro*. This expectation is confirmed by the experiments of Gey & Bang (30). Chick muscle fibroblasts in a plasma medium were destroyed within a week after inoculation. Cells lying deeply within the clot, however, and thus presumably escaping infection, remained unaffected. In a subsequent investigation Bang (31), therefore, propagated chick fibroblasts and chorioallantoic epithelial cells on films of polyvinyl formol (Formvar). Three strains of virus differing in virulence for chick embryos were employed. All three multiplied readily in both cell types. As revealed by electronmicrographs their effects on epithelial cells were, however, dissimilar. Certain of these elements exhibited long delicate projections from the surface which contained particles interpreted as partially and completely formed viral units. Disintegrative changes were noted deeper in the cell substance. Formation of filaments was more pronounced and the profounder degenerative changes less evident in cells inoculated with the strain of lowest virulence. Fibroblasts quickly disintegrated with the release of particles of virus and showed little or no tendency to form filaments at the surface. Fastier (32), using suspended cell cultures and subsequent explantation of the fragments in plasma clot, noted changes in cells from chick embryonic muscle which he stated differed in no way from those caused by poliomyelitis virus as described by Scherer & Syverton (33). Taking the cytopathogenic effect as criterion of viral growth he titrated suspen-

sions of the virus *in vitro* and found the results to compare favorably with those obtained by conventional animal inoculation methods.

Poliomyelitis viruses.—Evidence suggesting that poliomyelitis virus is cytopathogenic for extraneural tissue cells *in vitro* was obtained by Enders, Weller & Robbins (4) during the course of an investigation in which it was shown that a Type II strain could be cultivated readily under these conditions. Subsequent experiments by Robbins, Enders & Weller (34, 35) were confirmatory when it was found that representatives of Types I, II, and III viruses destroyed cells emigrating from human embryonic skin and muscle fragments in plasma roller tube cultures. Fibroblastic cells composing the greater part of the outgrowth became granular and lost their spindle shape, appearing as refractile spheres. Nuclear pyknosis was evident at this stage. Later the cells disintegrated, leaving behind masses of detritus. Examination of stained preparations of the infected outgrowth in such cultures has revealed little more than is to be seen in the natural state. It was then demonstrated that these manifestations of viral growth could be employed in infectivity titrations of infected materials by inoculating a series of cultures with increasing dilutions of the material and taking as the endpoint the highest dilution causing cellular degeneration. Infectivity titers measured *in vitro* corresponded reasonably well with those determined by animal inoculation. Furthermore, it was shown that homologous type specific immune serum, when mixed with the virus before addition to the culture, prevents the cytopathogenic effect. Since these results were described, cytopathic phenomena have been widely applied in studies on poliomyelitis. Not only have they provided the basis for the assay of virus and its antibody but also for the isolation of the agents from patients, for the study of viral mutants, for the preparation of complement fixing antigens and vaccines, for the testing of antibiotics, and for the determination of the effect of various chemical compounds on viral multiplication. Many of the reports on these applications of the technique have been recently reviewed (36). Reference to most of them will, therefore, not be made. Only investigations concerned directly with cytopathogenicity will be considered.

Contrary to expectation human and monkey cells, developing from a large variety of tissues, have been found susceptible to attack by poliomyelitis viruses. As yet the tissues of no other species of animal have been found susceptible (37, 38). In our laboratory (37, 39) it has been determined that cells emerging from the following human tissues are destroyed by one or more of the three prototype viruses: embryonic skin and muscle, intestine, brain, lung, kidney, amniotic membrane, and placenta (2 or 3 months gestation), postnatal prepuce, uterus, kidney, thyroid, thymus, and testis. Cells from human tonsils as Lepine and co-workers (40, 41) have reported, are likewise attacked by these viruses. Smith and his associates (42) and Syverton and his co-workers (43) showed that they could be cultivated in human and also in monkey testis. Later it was found that the spindle cells developing from these tissues were destroyed by virus. Morphologically pure strains of mon-

key testicular fibroblasts supported viral multiplication and exhibited essentially the same degenerative changes (44) as had been observed by Robbins *et al.* in cultures consisting largely of human fibroblastic elements. Renal epithelial cells of rhesus and cynomolgus monkeys are readily attacked and disintegrated by these viruses as Salk *et al.* (45) have pointed out. Because of the present widespread use of renal epithelial cells the changes induced in them by poliomyelitis virus merit particular description. At first a disturbance of the regular pavement-like arrangement, caused largely by the rounding of many units, is observed. Other cells become more refractile and much swollen, and in addition to the spheroidal shape may assume irregular and bizarre outlines. These alterations are quickly followed by loss of structural detail and final disintegration.

While certain cells from various tissues have been shown to support multiplication of the virus, it has not yet been determined how many other types may be involved. In part this uncertainty is dependent upon the generally recognized difficulty of distinguishing cell types and in part upon the impossibility of deciding whether certain types present in the original explant have or have not degenerated independently of viral influence. Until pure lines comparable to Earle's "L" strain of fibroblasts representing cells of different origins become available, complete assurance of the susceptibility of types other than epithelial cells and fibroblasts will be difficult to obtain. In this connection we may note that a strain of human carcinoma cells (HeLa) (8), almost surely consisting only of epithelial elements, has proved highly susceptible, and Stoler & Gey (46) have described the rapid cytolytic effect of virus on a strain (A-Fi) of human fibrosarcoma cells.

Differences in cell susceptibility have been recorded. Lepine & his coworkers (40) noted that fibroblasts from human tonsillar tissue were quickly affected by the three types of virus. Epithelial cells in the same cultures did not show cytopathic changes until later and then only a portion appeared to become involved. In cultures of human embryonic skin and muscle cells, we have repeatedly observed the same phenomena although eventually all the epithelial elements were destroyed. Wenner *et al.* (47) found that from 100 to 10,000 times more virus was required to produce cytopathic changes in monkey testicular cells than was necessary to obtain the same effect in monkey kidney cells. In unpublished experiments with Kibrick, we have determined that the difference in the minimal cytopathogenic dose of virus for monkey kidney cells is about 10 times greater than for human kidney cells. Similarly we have found that the minimal cytopathogenic dose for human uterine cells exceeds that for human kidney cells by a factor of 10 or more. Knowledge of such differences is of practical significance in evaluating procedures for the isolation of viruses and for the assay of infectivity and neutralizing antibodies. In this respect it is also important to note that the cells from monkeys immune to poliomyelitis have been shown by Ledinko & Melnick (48) to be as susceptible as those of normal animals. Since we have found (37) that the virus can destroy uterine cells from women between the ages of

40 and 65, some of whom with little doubt were immune to the disease, it is also probable that in human beings infection does not lead to cellular resistance.

The cytopathogenicity of poliomyelitis viruses has been studied by many workers in original tissue explants. Two other methods are available and are being increasingly employed. The first is indirect and depends upon the fact that acid production is reduced or arrested in infected cultures. The phenomenon was first observed in suspended cell cultures (34, 49). Evidence of viral multiplication is obtained by merely comparing the pH of the fluids in inoculated and uninoculated cultures after a suitable period of incubation. This method has been improved by Salk & Youngner (50) through the use of suspensions of monkey kidney cells obtained by Dulbecco's trypsinization procedure. By its means assay of virus and tests for neutralizing antibody can be conveniently and quickly carried out. The second method depends on the use of HeLa carcinoma cells or trypsinized cell suspensions (8, 51). In both instances the cell population can be accurately determined and used to prepare monolayer cultures directly on the glass. Cytopathic changes occur rapidly following inoculation of virus and are easily discerned. For special purposes, such as the analysis of factors involved in viral multiplication or the selection of mutants, Dulbecco's plaque counting method, which is applicable to poliomyelitis virus (51), doubtless will be much used.

Undifferentiated viruses from human feces.—During studies on the isolation of poliomyelitis virus *in vitro* from human feces Robbins and his associates (12) encountered a cytopathogenic agent in the stool of a case diagnosed as nonparalytic poliomyelitis which could not be identified. In subsequent unpublished investigations (52), it produced degeneration in cultures containing human embryonic fibroblasts, somewhat resembling that caused by poliomyelitis virus. However, the cells adjacent to the explant were first affected, whereas those at the periphery of the new growth were apt first to show the effect of poliomyelitis virus. Furthermore, as the process spread, all the available cells did not become involved. One observed rounded cells and fine cellular detritus lying between cells of normal appearance. Sera against poliomyelitis viruses did not prevent occurrence of these changes. Inoculation of suckling mice or monkeys produced no overt disease nor were lesions noted upon histological examination of the organs. We have isolated a number of agents with similar properties from fecal materials (52). In most instances they were derived from patients diagnosed as nonparalytic poliomyelitis. The development of neutralizing antibodies against the homologous viruses during the disease has been demonstrated *in vitro* in four cases. Riordan and his co-workers (10), who have so far contributed most to this subject, Duncan and his associates (14), and Monaci & Bonetti (53), have all described the isolation of agents with more or less comparable cytopathogenic properties which could not be identified as poliomyelitis or "C" viruses. Steigman & Kokko (54) have encountered a virus in human feces that first exerts its cytopathogenic effect at the periphery of the outgrowth.

Already enough has been learned to indicate that these agents are not

representatives of a single antigenic species. It cannot even be said that they form a related group. Before their relationships to each other and to disease in man can be defined, much additional data must be obtained. In accomplishing this, their cytopathogenic properties *in vitro* should be of much help.

Viruses from an epidemic exanthem.—From the feces of patients with an unidentified epidemic exanthem (55) that occurred in the area of Boston seven agents were isolated in tissue culture by Neva & Enders (56) which produced no disease in suckling mice. Their cytopathogenic properties were similar to certain of the undifferentiated fecal viruses. Since their effects on human cells were the same, and since antibodies developed during the illness which in cross neutralization tests suppressed cytopathic changes, it is probable that these "exanthem" viruses are representatives of the same species. It is also possible that they may be etiologically related to the disease. For these reasons they are here considered separately from the undifferentiated fecal viruses.

Cytopathogenicity was studied in roller tube cultures of human embryonic skin and muscle, postnatal foreskin, uterus, and kidney. Epithelial cells present in the foreskin and kidney cultures did not reveal definite alteration. Spindle shaped cells in all cultures were affected. Degeneration, which first appeared only after three days even when large quantities of virus were inoculated, usually began in the outgrowth adjacent to the explants, and then progressed slowly during a period of several days to a week until most of the cells were involved. Thus, their behavior contrasted sharply with that of poliomyelitis viruses. Rounding and subsequent formation of finely granular debris were the outstanding characteristics of the degenerative process. It is remarkable that while cultures of kidney tissue supported multiplication—indeed the original isolations were made in suspended cell cultures of this tissue—human renal epithelial cells showed no unmistakable signs of injury.

From a case resembling roseola infantum an agent was isolated in roller tube cultures by Neva & Enders (57) that induced a peculiar type of nuclear degeneration. Both epithelial and fibroblastic-like cells were affected with the former first exhibiting cytopathic change. Spindle cells became swollen; then followed a slowly progressive characteristic granulation of the contents of the nucleus which rendered the latter highly refractile. At first only occasional cells, distributed at random throughout the outgrowth, were visibly affected. Later, nearly all cells became involved and ultimately disintegrated. Renal and dermal epithelial cells showed rounded outlines and granulation of the nuclear substance and became markedly refractile. Suckling mice proved insusceptible and the patient from whom the agent was isolated developed neutralizing antibodies against it during his illness.

Viruses Causing Formation of Inclusion Bodies and Cell Degeneration

All of the agents assigned to this group have been reported to lead to the production in tissue cultures of either intranuclear or intracytoplasmic bod-

ies. In each instance evidence of viral multiplication other than the formation of inclusion bodies has been recorded.

Adenoid degeneration agents.—During the cultivation in roller tubes of adenoid tissues obtained from young children Rowe, Huebner and their coworkers (9) recently isolated and maintained in serial passage 14 agents with the general properties of viruses, all of which produced the same type of cell degeneration. The presence of these agents in a latent state in tissues of the respiratory tract is of interest and importance. In the original cultures, after varying periods, changes were observed in the sheet of epithelial cells surrounding the explant. Rounding of marginal cells was first seen, which progressed centripetally until all the outgrowth was involved. Complete degeneration occurred within 7 to 10 days. The appearance of affected cells was distinctive. They were "large, round, or slightly ovoid with a smooth distinct margin, clear peripheral cytoplasm, and a densely granular center, the nucleus obscured by the granulation." In preparations stained with hematoxylin-eosin eosinophilic swollen nuclei were present containing structures that appeared to be nuclear inclusions. These were usually basophilic but occasionally eosinophilic. The cytoplasm stained deep reddish purple and occasionally contained 10 to 12 small pale blue bodies. Often nucleus blended with cytoplasm to form a granular purplish mass. Cells from a variety of human embryonic and postnatal tissues showed cytopathogenic effects after inoculation of the agent. HeLa cells under its influence clumped, rounded, and separated from the glass. *In vitro* neutralizing antibodies were produced in rabbits following injection of the virus and were likewise demonstrated in the sera of certain normal persons and in gamma globulin.

Since this agent may be related to the acute respiratory virus described by Hilleman (7), on further study the latter may possibly be found to produce inclusion bodies. The peculiar nuclear changes in adenoidal epithelial cells resemble those induced by a virus isolated by Neva & Enders (57) from a case resembling roseola infantum. A comparative study, therefore, of the adenoidal agents and this virus might also be desirable.

Ectromelia virus.—This mouse virus, which is closely related to the vaccinia-variola group, regularly produces inclusions *in vivo*. Downie & McGaughey (58) in 1935 noted *in vitro* the appearance of intracytoplasmic eosinophilic bodies in infected embryonic and adult mouse tissues. In comparison with uninoculated controls that continued in excellent condition for 14 days, these authors observed that the cell outgrowth in infected cultures ceased by the seventh or eighth day, when signs of degeneration in the form of shrinking and vacuolization appeared. Unsuccessful attempts were made to cultivate the virus in chick embryonic tissues. The addition of specific antibody retarded but did not altogether prevent multiplication of virus and formation of inclusion bodies (59). Nauck (60) at about the same time propagated ectromelia virus in corneal epithelial cells of the rabbit, in which he noted inclusion bodies. Addition, however, of high concentrations of virus led to rapid degeneration without development of these structures.

Equine abortion virus.—This agent, which is probably limited in its host range to the horse, has recently been propagated in suspended cell cultures of equine spleen and lung by Randall *et al.* (61). Intranuclear eosinophilic inclusions were observed upon serial passage in these tissues, but only in primary cultures of hamster and chick embryonic cells. The excellent preservation of the equine tissues containing these bodies was emphasized. Accordingly, it is possible that this agent should not be placed among those that produce inclusions and cause cellular degeneration. We have done so with the reservation that, should future investigation fail to reveal destructive effects, it may take its place as the first prototype of a new group.

A curious fact is mentioned by Randall and co-workers (61) who state that inclusion bodies were not observed in cultures in which plasma was employed, for usually they are formed under these circumstances. Bang, Levey & Gey (62) failed to see them in plasma cultures of chick tissue in which multiplication of the virus of fowl pox occurred. The possible effect of plasma in suppressing inclusion body formation requires further investigation.

Fowl plague virus.—Hallauer (63) in an analysis of factors involved in multiplication was able to propagate this agent in Carrel flask cultures of chick embryo skin, brain, or iris epithelium. No growth occurred in cultures of fibroblasts, osteoblasts, or blood monocytes. The virus increased in the tissues of the pigeon, goose, and duck, but not in those of the rat or mouse. In susceptible cells morbid changes consisted of rounding and fatty degeneration. Beginning in the central area of cellular growth, the process ended in the formation of amorphous detritus. These phenomena recurred with great regularity and could be taken by an experienced observer as an index of the presence of virus. This early (1931) appreciation of the practical significance of cytopathic changes is noteworthy. Two years later Plotz & Ephrussi (64) also carried out a similar study of the virus in suspended cell cultures of chick embryonic tissues. Upon explanting fragments in plasma clots at daily intervals they showed that cellular outgrowth after two or three days exposure to the virus failed to occur, while fragments from control flasks were capable after a week or longer of producing cell outgrowth. They also reported (65) that mitosis ceased in inoculated cultures within 24 hr. after addition of virus, whereas in control cultures cell division continued. Additional evidence of a cytocidal action was afforded by experiments in which Plotz (66) found that fragments taken after three days from inoculated cultures failed to reduce methylene blue anaerobically in the light, whereas the dye was rapidly reduced by fragments from uninoculated cultures. Plotz apparently did not observe directly any morphologic changes.

Lately Flewett & Challice (67) have examined infected chick fibroblasts and epithelial cells by phase and electron microscopy. They described the formation of intranuclear inclusions, which seemingly arose through hypertrophy and granulation of the nucleolus. They described also the development of filaments and spherical bodies in the cytoplasm, considered to be the virus which, they suggest, may have escaped from the nucleus. These

phenomena were seen in cells of both types. Since pure strains of cells were not, however, employed, and since no titrations of viral infectivity were correlated with the morphological findings, it cannot be concluded that Hallauer was in error when he reported that fibroblasts failed to support multiplication.

Infectious canine hepatitis virus.—So far as is known, limitation to the natural host is also a characteristic of this agent. The recent success of Cabasso & his associates (68) in obtaining multiplication in cultures of dog kidney tissue, with resulting cellular destruction, affords another example of the particular value of the technique in cases where the host range is restricted. Renal epithelial cells rounded up and degenerated with changes similar to those produced in human or monkey cells by poliomyelitis virus. In contrast, fibroblasts in the same culture were unaffected. The virus failed to grow in normal chick embryonic tissues or in rabbit and monkey kidney. Cells from immune dogs exhibited degenerative changes comparable to those derived from susceptible animals, a finding comparable to that of Ledinko & Melnick (48) with poliomyelitis virus in cultures of immune monkey testis. Enough antigen emerged in the fluid of infected cultures to be employed in complement fixation tests with specific immune serum. Virus neutralization tests were carried out *in vitro*, taking the cytopathogenic effect as criterion of the endpoint. Although inclusion bodies were not described, Cabasso & Love (69) have subsequently observed them in infected canine kidney cultures.

Pseudorabies virus.—This agent was first cultivated by Traub (70) in suspended cell cultures of rabbit and guinea pig testicular tissue and chick embryonic tissues. Cell degeneration and the formation of eosinophilic intranuclear inclusions were described in about 60 per cent of rabbit tissue cultures. Materials from cultures of chick and guinea pig organs were not examined histologically. Sabin (71), in a study of the effect of immune serum on the virus, accepted the presence of inclusions as an index of infection in tissue culture. Recently Cserey-Pechany, Belady & Ivanovics (72) analyzed its cytopathogenicity more precisely. Using the explantation technique from suspended cell cultures of chick embryonic heart muscle, they demonstrated complete suppression of outgrowth with large viral inocula and with smaller quantities initial growth followed by subsequent degeneration. Serum of recovered swine prevented the cytopathogenic effect, a fact which subsequently formed the basis for the titration of antibody *in vitro* (73). Their findings differed from Traub's in that no inclusion bodies were seen in stained preparations, irrespective of the amount of virus inoculated. Explanation of the discrepancy is difficult, especially as Scherer (74) in cultures of Earle's L strain of mouse fibroblasts had no difficulty in observing them. Possibly in chick fibroblasts which were used exclusively by Ivanovics and his co-workers inclusion bodies do not develop. Analysis of this possible difference in response of chick and mammalian cells should be carried out.

Lymphogranuloma inguinale virus.—While this virus and the closely related psittacosis agent do not produce inclusion bodies of the "classical" ap-

pearance, infection of the cell results in the formation of dense intracytoplasmic bodies and of vesicles or vacuoles filled with granulocorpuscles or elementary bodies. Miyagawa & his associates (75) in mouse testicular and splenic tissues, Malamos (76), and Nauck (77) in rabbit corneal epithelium, and Gey & Bang (78) in a pure strain of human fibroblasts, have all described the formation of the vesicles. In the experiments of Malamos and Miyagawa they were seen within 8 to 72 hr. following inoculation of virus; Gey & Bang, who showed that infected cultures could be maintained for many weeks, never observed vesicles before the seventh day after infection. Malamos gives a clear description of the dense bodies formed most often in the early stages of infection which, unlike the violet-staining granulocorpuscles, appear blue with Giemsa. According to Malamos, within the dense bodies granulocorpuscles soon make their appearance. As the corpuscles increase in numbers with formation of the vesicle, the blue-staining matrix fragments and disappears. Gey & Bang do not allude to the blue-staining bodies. All these observers are agreed, however, that the virus does not cause rapid widespread injury to the cells. Indeed, Gey & Bang described the migration over a considerable distance of a cell containing a large vesicle; but they failed to see evidence of division in infected cells, which finally died with release of their burden of corpuscles.

There is much reason to believe that the vesicles with their granules seen in infected cells in tissue cultures represent collections of viral elementary bodies. The fact, however, that during a long period Gey & Bang were unable to demonstrate these structures while multiplication of virus continued, raises the question of whether at times the infective unit may assume another form or at least fail to cause the vesicular response. In this connection it is noteworthy that Sanders (79) did not find granulocorpuscles in cultures of guinea pig brain and chick embryonic tissue although viral increase occurred.

Psittacosis virus.—Since the pleomorphism exhibited by this virus suggested a developmental cycle Bedson & Bland (80) and Bland & Canti (81) employed tissue cultures to follow its intracellular development. They observed in infected mouse spleen or chick embryonic tissues early appearance of large densely staining cytoplasmic bodies which underwent metamorphosis into smaller masses to form eventually an aggregate of elementary bodies. The staining properties of these structures and the sequence of their development closely resemble those observed in cultures of lymphogranuloma venereum virus. In fact, although not always in interpretation, the findings of Bedson and his co-workers were confirmed by various other workers (82, 83, 84). Haagen & Crodel (83) cultivated psittacosis virus in various tissues of the rabbit and chick embryo. Large and small forms were seen only in epithelial and endothelial cells; other pathologic manifestations were not mentioned. Yanamura & Meyer (84) in a later study, however, concluded that in chick embryonic tissues fibroblasts were involved equally with other types. They also determined by smearing material from suspended cell cultures and by explanting fragments in plasma that widespread destruction of the cells oc-

curred on the fourth day after inoculation. Morgan & Wiseman (85) noted a decrease in tissue growth in roller tube cultures of infected chick tissue after increase of virus had occurred. This greater capacity of psittacosis virus to injure cells contrasts with the limited destructive effect of the lymphogranuloma venereum agent.

Vaccinia virus.—The first of the large number of studies on the behavior of vaccinia virus *in vitro* was carried out just before the era of tissue culture. Aldershoff & Broers (86) in 1906 inoculated rabbit corneas with the virus and kept them in a humid chamber at 37° C. Occasionally after 24 hr. and regularly after 48 hr. they observed the appearance of the cytoplasmic bodies of Guarnieri. In plasma coverslip perparations of rabbit cornea, Steinhardt & coworkers (87) obtained suggestive evidence of viral multiplication but failed to see definite "vaccine bodies" in the cells which retained their form for several weeks. Parker & Nye (88), who later demonstrated unequivocal viral proliferation, also failed to see inclusion bodies in rabbit testicular tissue or any other phenomena by which the presence of intracellular virus could be recognized. Rivers, Haagen & Muckenfuss (89) were the first to present satisfactory evidence for the development of Guarnieri bodies *in vitro*, which they found in epithelial cells of the rabbit's cornea. Inclusion bodies in cells of this type were not, however, observed by Rhodes & Van Rooyen (90) in pure cultures of epithelium from rabbit corneas. But in those consisting of all corneal layers they were seen in fibroblasts of the substantia propria. These authors also described Guarnieri bodies in chick embryo fibroblasts, a finding that was later confirmed by Feller, Enders & Weller (91). Bland & Robinow (92) studied the relationship of the elementary body to the inclusion body in cultures of rabbit's cornea and concluded that the latter represented a colony of elementary bodies. According to them, the inclusions occurred in epithelial cells. It seems, then, that in spite of this considerable amount of data, doubt remains concerning the type of cell involved in the formation *in vitro* of the inclusion body. More work with pure lines of cells will be required to settle the point. Probably, however, both fibroblasts and epithelial cells respond in this manner since reliable investigators have described Guarnieri bodies in both types.

A similar divergence appears among reports on the destructive effect of the virus. Steinhardt *et al.* (87), although noting accumulation of fat and granules in the cytoplasm of presumably infected cells, stated that they retained their form for several weeks even though not transferred to fresh plasma. Parker & Nye (88) did not observe any widespread degenerative changes that could be attributed to the virus. On the other hand, Maitland & Maitland (93) remarked on incipient degeneration after 24 hr. in chicken kidney fragments in suspended cell cultures which became extensive by the third day, but Rivers & his co-workers (94) using the same system, obtained outgrowth of cells from infected fragments three to five days after inoculation. Enders & Florman (95) demonstrated growth from chick embryo tissue that had been in the presence of the virus for at least 18 days, and Feller &

his associates (91), in roller tube cultures of chick embryonic tissues, showed that viral multiplication may continue for two months without gross cellular disintegration. In accord with Maitland's observations, on the other hand, are the following descriptions of cellular injury. Rivers *et al.* (89), in their experiments with rabbit cornea, witnessed the complete dissolution of the majority of the cells. Fischer (96) mentions experiments, without giving details, in which he inoculated "pure line" cultures of epithelial cells and fibroblasts with the virus and noted disintegration of the former after three days. The fibroblasts showed no changes. Haagen (97) failed to obtain multiplication of the virus in cultures of fibroblasts although growth occurred freely in rabbit testicular, renal, and pulmonary tissues. With these results, the findings of Feller *et al.*, mentioned above, are, however, not in agreement. Rous, McMaster & Hudack (98) refer to degeneration of rabbit fibroblasts, but Beard & Rous (99) saw no changes in pure cultures of Kupffer cells in which the virus actively multiplied. Recently Benedek & Kempe (100), in plasma cultures of chick embryonic and human embryonic skin and muscle tissue, have described granulation, rounding, and finally fragmentation of cells inoculated with vaccinia virus. Noyes (101) has succeeded in producing degenerative plaques in sheets of chick embryo cells. To reconcile these apparently contradictory results, systematic studies will be required in which various cell types from different species as well as different strains of virus (e.g., calf-adapted versus egg-adapted) will be systematically compared.

On the basis of available evidence we may tentatively conclude that the vaccinia virus, under certain conditions, induces inclusion body formation and leads to cell death while under others, these effects are not prominent, although active and continuing multiplication of virus may take place. As far as we are aware, there is no information concerning the growth of variola virus in tissue culture. Such experiments might reveal interesting differences between its behavior and that of vaccinia virus.

Cells from infected tissue cultures provide excellent material for the study of the elementary bodies of vaccinia, whether examined in stained preparations (102) or by electronmicroscopy (103).

Virus III.—With Virus III Andrewes carried out his classical experiments on the problem of acquired cellular immunity to viral infections. He first showed (104) that inoculation of cultures of normal rabbit testicular tissue was regularly followed by the appearance of intranuclear inclusions and multiplication of the virus. Subsequently (105), with the inclusion body as index of infection, he demonstrated complete susceptibility of cells from immune rabbits, provided they had been freed of neutralizing antibodies. When the latter were present, no infection of the cells occurred. With these observations as a basis, Andrewes proceeded to assay viral neutralizing antibody in immune serum. This was the first time, we believe, the tissue culture had thus been applied.

Shortly thereafter Topacio & Hyde (106) investigated changes occurring during the maturation of the inclusion body and observed eventual disinte-

gration of cells containing the bodies. Afterwards, in a significant study, Ivanovics & Hyde (107) analyzed the cytopathic effect of varying the viral inoculum. With large amounts, growth of cells was hindered or they were completely destroyed by the end of the fourth day. Under these conditions, no inclusions were seen. With smaller quantities, they regularly appeared in rabbit testicular cells but often only in limited areas of the outgrowth. Death of cells was again noted. In one respect the cytopathogenic effects of this agent are, as far as we know, unique among mammalian viruses. Following addition of large inocula no preliminary necrobiotic processes were noted in newly grown cells, such as pyknosis, karyorrhexis, etc. The cells merely burst, disintegrated, and autolyzed. Analogy with bacteria infected with phage is obvious. Inclusions formed in cells from other tissues, although never in numbers comparable to those in testicular cells. They were observed in a variety of cell types: fibroblasts, lymphocytes, leucocytes, histiocytes, and in the various kinds of endothelial-like cells. This wide range of susceptible tissues was not encountered by Andrewes (104) who failed to propagate the agent or to produce inclusions in cultures of tissue other than the testis. He remarked, however, that these tissues grew poorly, and this may have accounted for his failure. Paralleling the insusceptibility of other species are the findings that guinea pig testis (104) and chick embryonic tissues (107) did not support viral multiplication or inclusion-body formation. Also worthy of emphasis is the observation (107) that viral multiplication was greater in the testicular tissue of sexually mature rabbits than in that from immature individuals, a difference that likewise was exhibited in living animals.

Viruses Causing Formation of Multinucleated Cells or Syncytial Masses and Degeneration With or Without Inclusion Bodies

This group can be further subdivided into those agents which give rise to the formation of giant cells containing relatively few nuclei and those which characteristically produce large syncytial aggregates consisting of a common cytoplasmic matrix and numerous nuclei. The first subgroup includes the viruses of herpes simplex, zoster, and varicella; the second, agents recently isolated from cases of measles and those derived from renal tissues of apparently normal rhesus monkeys. As yet, the latter have been studied only in a preliminary manner.

Herpes simplex virus.—Parker & Nye (108) succeeded in maintaining herpes virus in plasma cultures of rabbit testis during 5 to 10 transfers. After several passages they observed that freshly added tissue grew poorly or not at all. They suggested that the virus might kill or injure fibroblasts. The development of intranuclear inclusions was not described. Similarly Gildemeister, Haagen & Schule (109) failed to find these structures in the same system although they carried the agent through 22 passages. Haagen, however, in another study with Rivers & Muckenfuss (89) regularly found inclusions in the epithelial cells of rabbit's cornea cultivated *in vitro*. They ap-

peared 24 to 48 hr. after inoculation when thickening of the epithelium, disappearance of intracellular bridges, swelling and rounding of the cells, and many amitotic giant cells were likewise seen. Inclusions occurred within the nuclei of the giant cells. Shortly afterwards Andrewes (110) described inclusions in the interstitial and germinal cells of both normal and immune rabbit and in normal guinea pig testis maintained in suspended cell cultures. A difference in cytopathogenicity between herpes simplex virus and Virus III was thus revealed, since the latter produces inclusion bodies only in interstitial cells (110).

Modern investigations have confirmed and extended these earlier findings. Scherer (74), with Earle's L strain of fibroblasts, noted inclusion bodies, amitotic giant cells, and a reduction in the cell population following inoculation of virus. In a comparative study of its effect on chick fibroblasts derived from different tissues (lung, heart, skeletal muscle, and cornea) and growing on cellophane sheets, Stulberg & Schapira (111) observed destruction of cells particularly in those originating from lung and cardiac tissues. These workers reported that cytopathogenicity was suppressed when plasma was employed. In unpublished experiments of our own, however, we have repeatedly observed rapid destruction of chick embryonic cells and various types of human cells emanating from original explants in plasma. Possibly the difference in results may depend upon their use of cells propagated in continuous culture, since these may be more deeply covered by the plasma and thus escape infection [Bang, Levey & Gey (62)]. Scott & his associates (112) have systematically followed the cellular changes during growth of the virus in rabbit corneal epithelial cells. Even during the latent period (2 to 6 hr. after inoculation), disturbances of nuclear elements were evident which became more pronounced during the phase of viral increase (6 to 12 hr.) until they terminated in the fully formed inclusion body. Shortly thereafter (16 hr.), the regular cellular arrangement became grossly disordered presenting "a fantastic picture" consisting of clumps of inclusion-body-bearing cells, naked nuclei, giant cells, ghost cells, and wide scattered pyknotic nuclei. Before this stage alteration of the cytoplasm occurred. The results of all these investigations, while illustrating the broad cytopathogenic spectrum of the virus emphasize the feature of giant cell formation which seems to distinguish it from the agents of Groups A and B.

Viruses of herpes zoster and varicella.—Because of their close similarity, if not identity, and their indistinguishable behavior in tissue culture, these two viruses will be considered together. Until recently, in spite of various reports of success, no satisfactory evidence had been presented to indicate that they could be cultivated *in vitro*. Weller first (113) in suspended cell cultures of human embryonic skin and muscle, and later (114) in roller tube cultures of this tissue and of human foreskin inoculated with vesicle fluid, noted eosinophilic intranuclear inclusion bodies closely resembling those found in the cutaneous lesions of the disease. Although in suspended cell cultures serial transmission of the agents responsible for inclusion body formation could not

be achieved, in roller tube cultures a number of strains have been maintained for many passages. Viruses from cases of varicella and herpes zoster have exhibited the same cytopathogenic properties. Serologic data supporting their etiologic relationship to the respective diseases, as well as indicating antigenic relationship or identity, have been obtained (115). Accompanying and following the maturation of the inclusion body, the cell exhibits degenerative changes consisting of rounding, increased refractility, and, finally, disintegration. Multinucleated giant cells are frequently formed. Fibroblasts and cutaneous epithelial cells undergo essentially the same changes following infection. A unique property of these viruses when propagated *in vitro* consists in their close association with the living cell. They are not released into the medium, at least in an infective state, and all attempts so far made to separate them from the cell have failed. Serial passage is most readily accomplished by the transfer of a few infected cells. These, settling on the surface of the new outgrowth, set up small foci of infection. As more cells become involved, the original focus enlarges and others are secondarily established until at last most of the available cell population becomes infected and eventually is destroyed. It would appear, therefore, that infection probably proceeds from cell to cell, only by direct contact. The capacity to form persisting foci of infected cells also appears to distinguish these agents. Although certain others such as herpes simplex virus (116) and Virus III (107) may initiate "focal lesions," these tend to become confluent, and the virus is liberated into the fluid and quickly infects the remaining cells.

Agents from measles.—Broadhurst & her associates (117) in 1938 described the development of inclusion bodies staining with Nigrosin in cultures of chick and human embryonic cells. Similar bodies were visualized in white blood cells and previously (118) in epithelial cells from swabbings of Koplik's spots and the nasal mucosa of acute measles cases. No other evidence was presented in support of the specific nature of these structures, and the observations were not confirmed by others.

Enders & Peebles (119) have lately isolated a number of agents all exhibiting the same properties in roller tube cultures of human renal cells inoculated with blood or throat washings from patients with measles. The cytopathic manifestations which become visible within two to seven days, depending on the size of the inoculum, consist in the formation of syncytial masses resembling large giant cells amid the sheets of epithelial cells. From 40 to 100 nuclei have been counted in these aggregates. Slight or moderate vacuolization of the cytoplasm is at first seen, which increases until the mass presents a torn or foamy appearance. Ultimately the elements composing the "giant cells" completely disintegrate. The process extends rather slowly, and it may require two weeks or more before all susceptible cells are involved. Fibroblasts apparently remain unaffected for long periods. In preparations fixed in Bouin's fluid and stained with hematoxylin and eosin the nuclei show striking changes. These consist in the presence of eosinophilic inclusions varying in size and margination of the chromatin. The bodies are surrounded

by a clear unstained zone and frequently two or more may be seen within the same nucleus, separated by chromatin "bridges." When formalin is used as fixative the clear zone is not seen, and the inclusion which stains less intensely is closely approximated to the nuclear chromatin. Taken together these changes comprise a reaction which is quite characteristic, in our experience, of these agents alone. Antibody which develops during the course of measles has been shown to prevent the cytopathogenic effects. This fact favors the hypothesis that they represent the etiologic agent of the disease.

Agents from renal tissues of rhesus monkeys.—During incubation of cultures of uninoculated monkey kidney cells, degenerative changes have been observed (120) which, in unstained preparations, could not be distinguished from those caused by the measles viruses. The responsible agents have been maintained *in vitro* in serial passages and, insofar as they have been studied, exhibit the properties of viruses. Inoculation of cultures of human kidney cells is also followed by formation of vacuolated syncytial masses. On staining either infected human or monkey cells, the changes induced by the monkey agents could be easily differentiated since the nuclear disturbances characteristic of the measles viruses were not present. Furthermore, serologic tests with sera from cases of measles have so far revealed no relationship between them.

VIRUSES OF AS YET ILL-DEFINED CYTOPATHOGENICITY *IN VITRO*

In the case of certain viruses the data regarding their cytopathogenic effects are insufficient to permit their assignment to the groups mentioned above. The reports concerning eight of them will, therefore, be separately reviewed.

Fowl pox virus.—The virus is discussed under this heading because it regularly produces inclusion bodies in its natural host and in chick embryos but has failed to do so when grown in chick embryo cells in roller cultures by Bang and his co-workers (62). In this respect it seems to have behaved in an exceptional manner, and we are inclined to believe that future investigations will show inclusion body formation *in vitro* following its multiplication under suitable conditions such, perhaps, as the avoidance of a plasma matrix. As recorded by Bang *et al.*, continuous growth occurred during a period of over 100 days in chick fibroblasts without the appearance of any abnormal cellular changes, but epithelial cells were killed by the virus.

Influenza virus.—In unpublished experiments Relova & Enders (1940) saw no cytopathic changes in roller tube cultures of chicken embryonic tissues although active multiplication of the influenza A virus (PR8 strain) was demonstrated. In suspended cell cultures of chick amniotic membranes Weller & Enders (121) also failed to note evidence of cell injury as indicated by alteration in acid production. Chick allantoic membranes were infected with PR8 and Lee B strains and maintained in suspended cell cultures by Eaton and his co-workers (122). When subsequently transferred to plasma

coated roller tubes, cellular outgrowth proceeded normally. Stulberg & Schapira (111), also using chick embryonic tissues growing on cellophane, obtained no increase of influenza A virus in fibroblasts derived from a variety of organs. In primary explants of lung and cornea, multiplication occurred, but no cellular changes were evident. Taylor (21) reported initial stimulation of cellular growth from fragments of infected chick allantoic membrane, although subsequently the cells appeared to die more quickly than those in control cultures. Pollard & Bussell (123), however, observed the partial destruction of cells from two human and two rodent tumors inoculated with a neurotropic strain of influenza A virus. In contrast, egg adapted virus did not exert a visible effect. Spherical and rod-like structures like those seen in allantoic fluid from infected chick embryos were observed by Murphy & collaborators (124) in electronmicrographs of chorioallantoic membrane infected with influenza viruses. Masses of elongated forms occurred in areas in which cells were destroyed. Whether destruction resulted from viral activity or other causes was not demonstrated. Evidently more investigation will be necessary, in which a variety of cells from different species are employed, before conclusions can be reached in respect to the cytopathogenic properties of this group.

Mumps virus.—In suspended cell cultures of chick amniotic membranes, where the multiplication of mumps virus may continue for several weeks, Weller & Enders (121) saw no indication of a depression of cellular metabolism. From such cultures Watson (125) explanted fragments of chick allantoic membrane into a plasma matrix at various intervals. The outgrowth of cells was comparable to that from uninoculated control fragments. Kilham & Murphy (126) cultivated the virus in cellophane-supported cells of the mouse embryo but saw no cellular changes ascribable to the virus. Contrary to these findings are those of Taylor (21) who described the failure of cell outgrowth in plasma explants from suspended cell cultures of chick amniotic membranes infected with mumps virus. Inspection of his data, however, reveals a wide variation in the number of uninoculated control explants developing cell outgrowth, a fact that demanded a statistical treatment of the results in order to reveal differences that were considered significant. Accordingly, repetition of these experiments seems desirable before his conclusion can be accepted that mumps exerts a cytopathogenic effect comparable to that of equine encephalomyelitis virus. Employing the fluorescent antibody technique of Coons, Watson (127) studied the intracellular position of the viral antigen in materials from cultures of various chick embryonic tissues. It was found only in the cytoplasm of cells that came in direct contact with the inoculum irrespective of the kind of tissue. No degenerative changes were described.

Rabies virus.—Although this agent has been repeatedly cultivated *in vitro*, it is doubtful that specific cytopathic changes have been observed. Barski & Maurin (128) recently reviewed the meager literature and conclude that inclusions resembling Negri bodies described by Borrel (129) were nonspecific since they also appeared in cultures inoculated with poliomyelitis

virus. Barski & Maurin, as well as Steinhardt and their co-workers (130) before them, found structures resembling Negri bodies in cultures of mouse or guinea pig brain tissue in both the presence and absence of rabies virus. Pollard & Bussell (123), in various tumor implants inoculated with the virus, concluded that it appeared to stimulate cell growth.

Rift Valley fever virus.—In infected mice this virus induces abundant formation of eosinophilic intranuclear inclusions, but in suspended cell cultures of chick embryonic tissues Saddington (131) did not find them, although multiplication of the agent seems to have occurred. He briefly mentioned the presence in stained sections of necrotic and normal-appearing cells lying side by side. Since some nonspecific cell degeneration occurs regularly in this type of culture, his observation cannot be accepted as proof of a viral cytonecrotic action. Here again is another agent whose cytopathogenicity requires further investigation, particularly in cultures of susceptible mammalian cells.

Salivary gland disease virus.—Andrewes (132) succeeded regularly in propagating this virus in cultures of salivary gland, ovary, and brain from the same species. He was unable, however, to cultivate the virus in serial passage in this medium. In this respect the agent behaves like varicella virus (113). Since the latter may, however, be propagated indefinitely in roller tube cultures, attempts to cultivate the salivary gland agent in the same manner might prove successful. Andrewes did not observe regular dissolution of the infected cells, although in one culture he refers to widespread tissue degeneration in which no inclusions were present but virus was demonstrated. The roller tube culture might afford a means of determining whether or not infection leads ultimately to cell death.

St. Louis encephalitis virus.—No changes attributable to viral activity were detected by Harrison & Moore (133) in suspended cell cultures of chick or mouse embryonic tissues inoculated with this virus. Its cultivation, however, was successful in these media over a period of four months. Huang (20) likewise discerned no morphological alterations in chick embryonic cells that were shown to support viral multiplication. However, in cells infected with St. Louis encephalitis virus, the agent of equine encephalomyelitis failed to grow or produce cytopathic changes. By taking advantage of this interfering effect, Huang (20) was able indirectly to titrate the activity of the former. It may be mentioned for the sake of completeness that in these same investigations Huang found that another virus, the so-called Jungeblut-Sanders mouse virus, behaved in the same manner as the St. Louis agent. Using mouse embryonic brain tissue Huang (19) also demonstrated impairment of glycolytic function in cultures infected with these viruses, although no other evidence of degeneration was presented. Neoplastic cells, however, may exhibit cytopathic changes, since in their human and murine tumor implants, Pollard & Bussell (123) witnessed disruption of outgrowth within 24 hr. and death of all elements at 48 hr. following inoculation of St. Louis encephalitis virus.

Yellow fever virus.—In plasma cultures of chick embryonic tissues, in

which the level of virus activity remained constant for 40 days, Fox (134) observed no degenerative changes. On the other hand Haagen (135), in cells of the rabbit's cornea and testis, described proliferative followed by degenerative alterations. The latter consisted in a rearrangement of chromatin material which eventually became acidophilic and granular. The appearance of the nucleus came to resemble that observed in infected animals. Haagen's observations seem sufficiently important to warrant attempts at confirmation, since if yellow fever virus produces recognizable changes *in vitro*, a convenient method would become available for the further study of its nature and properties.

Neoplastic changes induced by viruses.—Since Rous showed that a virus was responsible for the sarcoma in chickens that goes by his name, it has been recognized that certain agents of this class were capable of stimulating cells to increased proliferation resulting in the formation of tumors. Considering the possible significance of this fact for the problem of malignancy, it is surprising that during ensuing years few experiments on the effect of the tumor-producing viruses in tissue cultures have been carried out. The recorded results serve little more than to establish the facts that a few of these viruses can be grown in cultures containing appropriate varieties of cells and that in certain cases the latter may then seem to be rendered capable of causing tumors in the animal body. In most instances, however, the type of cell (epithelial, fibroblast, etc.) that is affected remains to be unequivocally determined.

Uncertainty regarding the type of cell involved lies at least partly in the fact that the elements supporting viral multiplication do not regularly exhibit decisive departures from the normal, either in respect to morphology or biological activity. Since it is probable that abnormalities of some sort must follow infection, the need for further investigations to define them is obvious.

The principal observations on the effects of tumor-producing virus on cells *in vitro* may be briefly summarized. Carrel (136) in his original studies on the Rous sarcoma in tissue culture noted the occurrence of two cell types: monocytes exhibiting amoeboid motion and fibroblasts. Tumor cells were usually strongly fibrinolytic as compared with normal cells. From early experiments with the Rous agent in cultures of normal monocytes and normal fibroblasts, Carrel tentatively concluded that the former alone supported its growth. Later findings, however, cast doubt on this conclusion since occasionally he succeeded in demonstrating the virus in cultures of fibroblasts after prolonged cultivation (137). To be emphasized is the fact that after infection normal cells often exhibited enhanced fibrinolytic capacity. It is also of especial interest that Carrel & Ebeling (138) observed the occurrence of more rapid death of the infected cells. Ludford (139), working with the Fujinami as well as the Rous sarcoma virus in cultures of chicken fibroblasts and monocytes, obtained evidence suggesting that the former and not the latter were involved in viral multiplication. He failed, however, to discern

any definite morphological changes characteristic of infection and, therefore, was unable to prove that the infected fibroblasts were actually converted into sarcoma cells. In contrast, Halberstaedter and co-workers (140) described alterations in chick embryonic heart muscle infected with the Rous virus after 3 to 5 subcultures. These consisted in the emergence of round cells among the fibroblastic sheets and ultimately in the development of typical Rous "colonies" as described by Fischer (141) consisting of highly polymorphic cells and exhibiting an increased capacity to liquefy the clot. The virus-bearing cells in the colonies died after a time unless normal tissue fragments were added. In a recent well-controlled study, Sanford and her associates (142) have confirmed these earlier observations on the role of the fibroblast in the growth of Rous virus but make no mention of any criteria whereby the infected cell can be distinguished *in vitro*.

Few attempts to propagate other tumor-producing viruses in tissue culture have been made. Although several investigators succeeded in maintaining the agent of myxomatosis throughout many passages in suspended cell cultures of rabbit testicular tissue, histological changes were not described. Faulkner & Andrewes (143) cultivated an "inflammatory" strain of rabbit fibroma virus (Shope) in cultures of the same tissue. They failed, however, to distinguish any definite effect of the virus. In spite of repeated attempts, these workers obtained no evidence that a fibromatous strain of the virus (i.e., one causing proliferation rather than inflammatory reactions) multiplied in rabbit testicular tissue.

Coman (144) inoculated cultures of normal rabbit skin with Shope's papilloma virus and noted increased proliferation of epithelial cells. When these were introduced into the liver of the animal supplying the skin, a tumor was produced. The virus alone or normal epithelial cells did not have this effect. Multiplication of the agent *in vitro* was not demonstrated by serial passages. Doljanski & Pikovski (145) cultivated a strain of fowl leucosis virus in normal chick fibroblasts and other tissues. The presence of the leukotic agent caused no recognizable changes in the cells.

It would seem that the time was propitious for an intensive renewal of the study of this class of viruses, especially for the purpose of discovering means of recognizing their presence *in vitro*. Such investigations might well involve the use not only of the phase microscope but also the application of the Coons' fluorescent antibody technique for the localization of antigen, and, possibly, interference of the cytopathogenic effect of other viruses.

FINAL COMMENT

This review, it is hoped, may have fulfilled its primary purpose of acquainting the reader with the present status of knowledge concerning the capacity of most of the mammalian viruses to injure cells in tissue cultures. The attempt has been made not only to indicate certain of the more important data that have been already accumulated but also to define areas in which information is lacking, fragmentary, or unreliable. While it has lately

become increasingly apparent that the growth of many viruses can be recognized and so accurately measured by the effects they produce on cells, it is equally evident that only a beginning has been made in the exploration of the many problems concerned with viral cytopathogenicity that can be investigated by means of the tissue culture. It would seem that progress in the future will greatly depend upon the development of pure strains of various cell types, derived from representative species and exhibiting constant susceptibility to infection. With such strains it should be possible eventually to formulate a systematic account of the cytopathology of viruses expressed under the simple conditions provided by the tissue culture, as contrasted with the more complex environment of the living animal. Such an account will almost certainly include analyses of functional as well as morphologic manifestations of cellular injury. Finally, it is safe to predict that with the advancement of knowledge will come, as always, additional contributions to the practical problems of etiologic identification, laboratory diagnosis, and the control of disease.

LITERATURE CITED

1. Sanders, M., Kiem, I., and Lagunoff, D., *Arch. Pathol.*, **56,** 148–225 (1953)
2. Robbins, F. C., and Enders, J. F., *Am. J. Med. Sci.*, **220,** 316–38 (1950)
3. Moore, A., *Ann. Rev. of Microbiol.*, **8,** 393–410 (1954)
4. Enders, J. F., Weller, T. H., and Robbins, F. C., *Science*, **109,** 85–87 (1949)
5. Lynn, J. W., and Morgan, H. R., *Arch. Pathol.*, **57,** 301–16 (1954)
6. Womack, C. R., and Kass, E. H., *J. Immunol.*, **71,** 152–67 (1953)
7. Hilleman, M. R., and Werner, J. H., *Proc. Soc. Exptl. Biol. Med.*, **85,** 183–88 (1954)
8. Scherer, W. F., Syverton, J. T., and Gey, G. O., *J. Exptl. Med.*, **97,** 695–709 (1953)
9. Rowe, W. P., Huebner, R. J., Gilmore, L. K., Parrott, T. H., and Ward, T. G., *Proc. Soc. Exptl. Biol. Med.*, **84,** 570–73 (1953)
10. Riordan, J. T., Ledinko, N., and Melnick, J. L., *Am. J. Hyg.*, **55,** 339–46 (1952)
11. Stulberg, C. S., Schapira, R., and Eidam, C. R., *J. Immunol.*, **72,** 107–11 (1954)
12. Robbins, F. C., Enders, J. F., Weller, T. H., and Florentino, G. L., *Am. J. Hyg.*, **54,** 286–93 (1951)
13. Weller, T. H., Robbins, F. C., Stoddard, M. B., and Florentino, G. L., *J. Immunol.*, **71,** 92–97 (1953)
14. Duncan, D., Silverthorne, N., McNaughton, G. A., Johnson, C. C. R., and Rhodes, A. J., *Can. J. Public Health*, **45,** 55–63 (1954)
15. Godman, G. C., Bunting, H., and Melnick, J. L., *Am. J. Pathol.*, **28,** 223–57 (1952)
16. Gajdusek, D. C., Anslow, R. O., Hubbell, E. J., and Yager, R. H., *J. Immunol.*, **72,** 224–28 (1954)
17. Huang, C. H., *Proc. Soc. Exptl. Biol. Med.*, **51,** 396–98 (1942)
18. Huang, C. H., *J. Exptl. Med.*, **78,** 111–26 (1943)
19. Huang, C. H., *Proc. Soc. Exptl. Biol. Med.*, **54,** 160–61 (1943)
20. Huang, C. H., *Proc. Soc. Exptl. Biol. Med.*, **54,** 158–60 (1943)
21. Taylor, C. E., *J. Immunol.*, **71,** 125–33 (1953)

22. Bang, F. B., and Gey, G. O., *Bull. Johns Hopkins Hosp.*, **91,** 427–49 (1952)
23. Dulbecco, R., *Proc. Nat. Acad. Sci. U. S.*, **38,** 747–52 (1952)
24. Dulbecco, R., and Vogt, M., *J. Exptl. Med.*, **99,** 183–99 (1954)
25. Smith, W. M., and Evans, C. A., *J. Immunol.*, **72,** 353–59 (1954)
26. Striegler, E., *Zentr. Bakt. Parasitenk., Abt. I, Orig.*, **128,** 332–36 (1933)
27. Köbe, K., and Fertig, H., *Zentr. Bakt. Parasitenk., Abt. I, Orig.*, **138,** 14–20 (1937)
28. Hecke, F., *Zentr. Bakt. Parasitenk., Abt. I., Orig.*, **116,** 386–414 (1930)
29. Frenkel, H. S., *Am. J. Vet. Res.*, **10,** 142–145 (1949)
30. Gey, G. O., and Bang, F. B., *Trans. N. Y. Acad. Sci.*, **14,** 15–24 (1951)
31. Bang, F. B., *Bull. Johns Hopkins Hosp.*, **92,** 291–308 (1953)
32. Fastier, L. B., *J. Immunol.*, **72,** 341–47 (1954)
33. Scherer, W. F., and Syverton, J. T., *J. Exptl. Med.*, **96,** 389–400 (1952)
34. Robbins, F. C., Enders, F. J., and Weller, T. H., *Proc. Soc. Exptl. Biol. Med.*, **75,** 370–74 (1950)
35. Enders, J. F., *Poliomyelitis. Papers and Discussions Presented at the Second International Poliomyelitis Conference*, 33–43 (J. B. Lippincott Co., Philadelphia, 555 pp., 1952)
36. Weller, T. H., *New Eng. J. Med.*, **249,** 186–95 (1953)
37. Robbins, F. C., Weller, T. H., and Enders, J. F., *J. Immunol.*, **69,** 674–94 (1952)
38. Evans, C. A., Byatt, P. H., Chambers, V. C., and Smith, W. M., *J. Immunol.*, **72,** 348–52 (1954)
39. Weller, T. H., Robbins, F. C., and Enders, J. F. (Unpublished observations)
40. Lepine, P., Barski, G., de Brion, G., and Monaci, V., *Compt. rend.*, **236,** 154–55 (1953)
41. Barski, G., Lepine, P., Monaci, V., and de Brion, G., *Ann. Inst. Pasteur*, **84,** 825–40 (1953)
42. Smith, W. M., Chambers, V. C., and Evans, C. A., *Proc. Soc. Exptl. Biol. Med.* **76,** 696–700 (1951)
43. Syverton, J. T., Scherer, W. F., and Butorac, G., *Proc. Soc. Exptl. Biol. Med.*, **77,** 23–28 (1951)
44. Scherer, W. F., and Syverton, J. T., *J. Exptl. Med.*, **96,** 389–400 (1952)
45. Salk, J. E., Bennett, B. L., Lewis, L. J., Ward, E. N., and Youngner, J. S., *J. Am. Med. Assoc.*, **151,** 1081–98 (1953)
46. Stoler, M., and Gey, M. K., *Bull. Johns Hopkins Hosp.*, **92,** 385–87 (1953)
47. Wenner, H. A., Miller, C. A., Kamitsuka, P., and Wilson, J. C., *Am. J. Hyg.*, **59,** 221–35 (1954)
48. Ledinko, N., and Melnick, J. L., *Proc. Soc. Exptl. Biol. Med.*, **81,** 144–48 (1952)
49. Weller, T. H., Enders, J. F., Robbins, F. C., and Stoddard, M. B., *J. Immunol.*, **69,** 645–71 (1952)
50. Salk, J. E., and Youngner, J. S., *Applications of Tissue Culture Methods in the Study of Viral Infections* (Presented in Symposium before 5th Meeting Soc. Am. Bacteriologists, 1954)
51. Dulbecco, R., and Vogt, M., *J. Exptl. Med.*, **99,** 167–82 (1954)
52. Kibrick, S., Robbins, F. C., and Enders, J. F. (Unpublished observations)
53. Monaci, V., and Bonetti, F., *Boll. ist. sieroterp. milan.*, **33,** 3–4 (1954)
54. Steigman, A. J., and Kokko, U. P., *Federation Proc.*, **12,** 461 (1953)
55. Neva, F. A., Feemster, R. F., and Gorbach, I. J., *J. Am. Med. Assoc.*, **155,** 544–48 (1954)

56. Neva, F. A., and Enders, J. F., *J. Immunol.*, **72,** 307–14 (1954)
57. Neva, F. A., and Enders, J. F., *J. Immunol.*, **72,** 315–21 (1954)
58. Downie, A. W., and McGaughey, C. A., *J. Pathol. Bact.*, **40,** 147–59 (1935)
59. Downie, A. W., and McGaughey, C. A., *J. Pathol. Bact.*, **40,** 297–310 (1935)
60. Nauck, E. G., *Arch. exptl. Zellforsch.*, **18,** 336–42 (1935)
61. Randall, C. C., Ryder, F. W., Doll, E. R., and Schell, F. S., *Am. J. Pathol.*, **29,** 139–53 (1953)
62. Bang, F. B., Levey, E., and Gey, G. O., *J. Immunol.*, **66,** 329–45 (1951)
63. Hallauer, C., *Z. Hyg. Infektions Krankh.*, **113,** 61–74 (1931)
64. Plotz, H., and Ephrussi, B., *Compt. rend. soc. biol.*, **112,** 525–26 (1933)
65. Plotz, H., and Ephrussi, B., *Compt. rend. soc. biol.*, **113,** 711–12 (1933)
66. Plotz, H., *Compt. rend. soc. biol.*, **118,** 1400–1 (1935)
67. Flewett, T. H., and Challice, C. E., *J. Gen. Microbiol.*, **5,** 279–86 (1951)
68. Cabasso, V. J., Stebbins, M. R., Norton, T. W., and Cox, H. R., *Proc. Soc. Exptl. Biol. Med.*, **85,** 239–45 (1954)
69. Cabasso, V. J., and Love, R. (Personal communication to author)
70. Traub, E., *J. Exptl. Med.*, **58,** 663 (1933)
71. Sabin, A. B., *Brit. J. Exptl. Pathol.*, **16,** 84–101 (1935),
72. Cserey-Pechany, E., Belady, I., and Ivanovics, G., *Acta Physiol. Acad. Sci. Hung.*, **2,** 229–42 (1951)
73. Ivanovics, G., Koch, A., and Cserey-Pechany, E., *Acta Physiol. Acad. Sci. Hung.*, **4,** 383–99 (1953)
74. Scherer, W. F., *Am. J. Pathol.*, **29,** 113–38 (1953)
75. Miyagawa, Y., Mitamura, T., Yaoi, H., Ischii, N., Okanishi, J., Goto, T., and Shimiga, S., *Japan. J. Exptl. Med.*, **14,** 207–19 (1936)
76. Malamos, B., *Zentr. Bakt. Parasitenk., Abt. I, Orig.*, **143,** 1–7 (1938)
77. Nauck, E. G., *Arch. Schiffs- u. Tropen-Hyg.*, **41,** 748 (1937); cited by Lynn, J. W., and Morgan, H. R., *Arch. Pathol.*, **57,** 301–16 (1954)
78. Gey, G. O., and Bang, F. B., *Bull. Johns Hopkins Hosp.*, **65,** 393–417 (1939)
79. Sanders, M., *J. Exptl. Med.*, **71,** 113–28 (1940)
80. Bedson, S. P., and Bland, J. O. W., *Brit. J. Exptl. Pathol.*, **13,** 461–66 (1932)
81. Bland, J. O. W., and Canti, R. G., *J. Pathol. Bact.*, **40,** 231–41 (1935)
82. Levinthal, W., *Lancet*, **1,** 1207–10 (1935)
83. Haagen, E., and Crodel, B., *Zentr. Bakt. Parasitenk., Abt. I, Orig.*, **138,** 20–27 (1936)
84. Yanamura, H. Y., and Meyer, K. F., *J. Infectious Diseases*, **68,** 1–15 (1941)
85. Morgan, H. R., and Wiseman, R. W., *J. Infectious Diseases*, **79,** 131–33 (1946)
86. Aldershoff, H., and Broers, C. M., *Ann. Inst. Pasteur*, **20,** 779–84 (1906)
87. Steinhardt, E., Israeli, C., and Lambert, R. A., *J. Infectious Diseases*, **13,** 294–300 (1913)
88. Parker, F., Jr., and Nye, R. N., *Am. J. Pathol.*, **1,** 325–35 (1925)
89. Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exptl. Med.*, **50,** 665–72 (1929)
90. Rhodes, A. J., and Van Rooyen, C. E., *J. Pathol. Bact.*, **45,** 253–56 (1937)
91. Feller, A. E., Enders, J. F., and Weller, T. H., *J. Exptl. Med.*, **72,** 367–88 (1940)
92. Bland, J. O. W., and Robinow, C. F., *J. Pathol. Bact.*, **48,** 381–403 (1939)
93. Maitland, H. B., and Maitland, M. C., *Lancet, II,* 596–97 (1928)
94. Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exptl. Med.*, **50,** 181–87 (1929)

95. Enders, J. F., and Florman, A. L., *Proc. Soc. Exptl. Biol. Med.*, **49,** 153–55 (1942)
96. Fischer, A., *Gewebezuchtung. Handbuch der Biologie der Gewebezellen in vitro*, 3rd ed. (R. Muller u. Steinicke, München, Germany, 1930)
97. Haagen, E., *Zentr. Bakt. Parasitenk., Abt. I., Orig.*, **120,** 304–10 (1931)
98. Rous, P., McMaster, P. D., and Hudack, S. S., *J. Exptl. Med.*, **61,** 657–88 (1935)
99. Beard, J. W., and Rous, P., *J. Exptl. Med.*, **67,** 883–910 (1938)
100. Benedek, A. W., and Kempe, C. H., *Proc. Soc. Exptl. Med.*, **82,** 520–23 (1953)
101. Noyes, W. F., *Proc. Soc. Exptl. Biol. Med.*, **83,** 426–29 (1953)
102. Haagen, E., and Kodama, M., *Zentr. Bakt. Parasitenk., Abt. I, Orig.*, **133,** 23–32 (1934)
103. Wirth, J., and Athanasiu, P., *Proc. Soc. Exptl. Biol. Med.*, **70,** 59–61 (1949)
104. Andrewes, C. H., *Brit. J. Exptl. Pathol.*, **10,** 188–90 (1929)
105. Andrewes, C. H., *Brit. J. Exptl. Pathol.*, **10,** 273–80 (1929)
106. Topacio, T., and Hyde, R. R., *Am. J. Hyg.*, **15,** 99–124 (1932)
107. Ivanovics, G., and Hyde, R. R., *Am. J. Hyg.*, **23,** 55–73 (1936)
108. Parker, F. Jr., and Nye, R. N., *Am. J. Pathol.*, **1,** 337–40 (1925)
109. Gildemeister, E., Haagen, E., and Schule, L., *Zentr. Bakt. Parasitenk., Abt. I, Orig.*, **114,** 309–14 (1929)
110. Andrewes, C. H., *J. Pathol. Bact.*, **33,** 301–12 (1930)
111. Stulberg, C. S., and Schapira, R., *J. Immunol.*, **70,** 51–59 (1953)
112. Scott, T. F. McNair, Burgoon, C. F., Coriell, L. L., and Blank, H., *J. Immunol.*, **71,** 385–96 (1935)
113. Weller, T. H., and Stoddard, M. B., *J. Immunol.*, **68,** 311–19 (1952)
114. Weller, T. H., *Proc. Soc. Exptl. Biol. Med.*, **83,** 340–46 (1953)
115. Weller, T. H. (Unpublished observations)
116. Enders, J. F. (Unpublished observations)
117. Broadhurst, J., Cameron, G., and Saurino, V., *J. Infectious Diseases*, **62,** 6–20 (1938)
118. Broadhurst, J., Maclean, M. E., and Saurino, V., *J. Infectious Diseases*, **61,** 201–7 (1937)
119. Enders, J. F., and Peebles, T. C., *Proc. Soc. Exptl. Biol. Med.*, **86,** 277–86 (1954)
120. Peebles, T. C., Cheatham, W. J., and Enders, J. F. (Unpublished observations)
121. Weller, T. H., and Enders, J. F., *Proc. Soc. Exptl. Biol. Med.*, **69,** 124–28 (1948)
122. Eaton, M. D., Cheever, F. S., and Levenson, C. G., *J. Immunol.*, **66,** 463–75 (1951)
123. Pollard, M., and Bussell, R. H., *Proc. Soc. Exptl. Biol. Med.*, **80,** 574–78 (1952)
124. Murphy, J. S., Karzon, D. T., and Bang, F. B., *Proc. Soc. Exptl. Biol. Med.*, **73,** 596–99 (1950)
125. Watson, B. (Personal communication to author)
126. Kilham, L., and Murphy, H. W., *Proc. Soc. Exptl. Biol. Med.*, **80,** 495–98 (1952)
127. Watson, B., *Proc. Soc. Exptl. Biol. Med.*, **79,** 222–24 (1952)
128. Barski, G., and Maurin, J., *Ann. Inst. Pasteur*, **78,** 411–13 (1950)
129. Borrel, A., *Bull. Soc. franç. dermatol. syphilig.*, **37,** 1259 (1930); cited by Barski, G., and Maurin, J., *Ann. Inst. Pasteur*, **78,** 411–13 (1950)
130. Steinhardt, D., Poor, W., and Lambert, R. A., *J. Infectious Diseases*, **11,** 459–63 (1912)
131. Saddington, R. S., *Proc. Soc. Exptl. Biol. Med.*, **31,** 693–94 (1934)
132. Andrewes, C. H., *Brit. J. Exptl. Pathol.*, **11,** 23–34 (1930)
133. Harrison, R. W., and Moore, E., *Proc. Soc. Exptl. Biol. Med.*, **35,** 359–61 (1936)

134. Fox, J. P., *Am. J. Hyg.*, **46**, 1–20 (1947)
135. Haagen, E., *Arch. exp. Zellforsch. Gewebezücht.*, **15**, 405–21 (1934)
136. Carrel, A., *J. Am. Med. Assoc.*, **84**, 157–58 (1925)
137. Carrel, A., J. Exptl. Med., **43**, 647–68 (1926)
138. Carrel, A., and Ebeling, A. H., *J. Exptl. Med.*, **43**, 461–68 (1926)
139. Ludford, R. J., *Am. J. Cancer*, **31**, 414–29 (1937)
140. Halberstaedter, L., Doljanski, L., and Tenenbaum, E., *Brit. J. Exptl. Pathol.*, **22**, 179–87 (1941)
141. Fischer, A., *Gewebezuchtung* (Muller u. Steinicke, München, Germany, 1936) cited by Halberstaedter, L., Doljanski, L., and Tenenbaum, E., *Brit. J. Exptl. Pathol.*, **22**, 179–87 (1941)
142. Sanford, K. K., Likely, G. D., Bryan, R. W., and Earle, W. R., *J. Nat. Cancer Inst.*, **12**, 1317–44 (1951)
143. Faulkner, G. H., and Andrewes, C. H., *Brit. J. Exptl. Pathol.*, **16**, 271–75 (1935)
144. Coman, D. R., *Cancer Research*, **6**, 602–7 (1946)
145. Dolianski, L., and Pikovski, M., *Cancer Research*, **2**, 626–31 (1942)

AUTHOR INDEX

A

Abbey, A., 171, 172
Abel, W., 267
Abelson, P.H., 80,95
Abinanti, M.M., 430
Abraham, E.P., 167,171,172, 185
Abrams, A., 424
Abrams, R., 375
Abreu, L.A., 51
Acker, R.F., 172
Ackermann, W., 402
Ackermann, W.W., 378,383
Adams, A.R.D., 284
Adams, G., 284
Adams, M.E., 107,109,111
Adams, M.H., 194,199,239
Adams, S.L., 462,464
Adelberg, E.A., 97,182,197
Adler, F.L., 367
Adriano, F.J., 454
Agarwal, P.N., 452,454,460, 462,464
Ahern, J.J., 170,259,261,262
Ahmad, A., 210
Ainsworth, G.C., 258
Ajl, S.J., 80,118,119
Akanabe, S., 172
Albert, A., 170
Albrecht, H., 451
Albrecht, R.M., 298
Albrecht, T.W., 437
Albrink, M.J., 294
Alderman, I.M., 168
Aldershoff, H., 488
Alexander, H., 434
Alexander, H.E., 168,186,209
Alexander, J.D., Jr., 264
Alexander, M., 184
Alff, Y., 442
Allain, D.S., 280
Allan, M.B., 92
Allen, J.G., 298
Almquist, H.J., 357
Alonso, D., 385
Alston, J.M., 142
Altenbern, R.A., 93,251
Altschul, A., 296
Altschul, R., 379
Alvares Corréa, M.O., 142
Alvarez, D.A., Jr., 379
Ambrus, C.M., 167
Ames, A.M., 382
Ames, B.N., 182
Amsz, J., 464
Anchel, M., 172
Anchezar, B.V., 141
Anderson, A.A., 167
Anderson, A.W., 436
Anderson, C.G., 419,421
Anderson, E.A., 261
Anderson, E.H., 220
Anderson, E.O., 443
Anderson, E. S., 194, 205, 239
Anderson, G.W., 265,266,267, 459
Anderson, H.H., 280, 281
Anderson, L., 83
Anderson, L.G., 449,450
Anderson, P.A., 224
Anderson, R.F., 167
Anderson, S.G., 312,316
Anderson, T.E., 134
Anderson, T.F., 7,194,199, 237,238,241
Anderson, W.A.D., 279
Andreasen, A.A., 449,450,462
Andrew, V.M., 368
Andrewes, C.H., 290,312,313, 315,322,323,324,325,326, 400,489,490,491,495,497
Andrews, W.H. Horner, 278, 282
Angelico, R., 7
Anigstein, L., 375
Anslow, R.O., 477
Appel, P.P., 267
Appelbaum, E., 296
Appert, J., 106
Appleby, J.C., 203,312,320, 324
Appleyard, R.K., 204,205,249
Arana, A.J., 358
Archetti, I., 138,318
Arima, K., 184
Aristovskaya, T.V., 450,452
Arkani, M., 124
Armas-Cruz, R., 294
Armen, D.M., 346
Armitage, P., 221
Armstrong, F.L., 261
Arnaki, M., 458
Arndt, U.W., 217
Arnold, W., 125
Arnstein, H.R.V., 80
Arrazola, J.M., 462
Arzt-Hofbauer, 296
Asahina, T., 172
Asano, M., 458
Asano, N., 56,169
Aschan, K., 193,215
Ascoli, M., 160
Ashton, G.C., 265,266,267
Asnis, R.E., 11,83
Asselineau, J., 124
Astbury, W.T., 6,321
Athanasiu, P., 489
Atkeson, F.W., 267
Atkinson, R.L., 267
Atwood, K., 25,26
Atwood, K.C., 199,222
Aubert, J.-P., 77,78
Augier, J., 107
Austrian, R., 208,209,252
Austwick, P.K.C., 258
Avakian, S., 55
Avant, W.H., 281
Avi-Dor, Y., 188,216
Awad, F.I., 156,158
Ayengar, P., 58,93

B

Baalsrud, K., 108,111,112,113, 115,116
Baalsrud, K.S., 111,112,115
Babel, F.J., 434,435,441
Babudieri, B., 134,136,138, 144,145,146
Bacchus, H., 382
Bachofer, C.S., 219,220
Backus, M.P., 167
Bacon, G.A., 378
Bacon, J.S.D., 84
Baer, B.S., 239
Baile, D.L., 50
Bailey, G.H., 381
Bailey, L., 172
Bailey, M.L., 326
Baix, 404,405
Baker, B.R., 172
Baker, L.A., 170,173
Baker, M., 273
Baker, R.F., 8,10,25
Baker, W., 172
Bakon, B., 357
Bale, W.F., 337,347
Balis, M.E., 62,187
Ballard, M.B., 161
Ballarin, O., 429
Ballentine, R., 54,197
Bang, F.B., 367,403,478,479, 485,487,491,493
Banks, M., 244
Bant, F.B., 494
Barahona, R., 292
Barban, S., 80
Barber, F.W., 50,430,432
Barber, M., 183,187,188
Barber, R.S., 266,267
Barclay, A.E., 278,284
Bard, R.D., 80
Bardach, M., 394
Bardos, T.J., 51,53
Barenberg, L.H., 296,297
Baribo, L.E., 441
Baris, Y., 442
Barker, H.A., 93,94,95,105, 106,107,109,121
Barksdale, W.L., 185,205
Barkulis, I.L., 168
Barkulis, S.S., 88,89

Barlow, J.L., 237
Barnabei, O., 63
Barnes, J. M., 383, 413
Barnett, G., 312, 326
Barnett, R.N., 298
Baron, L. S., 208, 252
Barondess, J. A., 296, 297
Barr, M., 411, 414, 415, 421, 422
Barratt, R.V., 210
Barratt, R.W., 212
Barrett, J. T., 80, 194
Barrington, L. F., 240
Barron, E.S.G., 298
Barski, G., 480, 481, 494
Barsky, J., 169
Bartholomew, J. W., 8, 9
Bartholemew, W.H., 464
Bartley, E. E., 267
Bartmann, K., 222
Barton, D. H. R. , 172
Barton-Wright, E. C., 453
Barzelatto, J., 292
Bashe, W. J., Jr., 297
Bass, A., 451
Bassen, F. A., 298
Bassett, H. J., 433
Bassham, J. A., 120
Batchelor, W.H., 335
Bateman, J. B., 224
Batista, S., 162, 163
Baumann, A., 109
Baumann, C.A., 53, 265, 266
Bautz, E., 190
Bayan, A. P., 171
Baylet, R., 285
Beadenkopf, W.G., 298
Beale, G. H., 193
Bealing, F. J., 84,85
Bean, W. B., 294
Beard, J. W., 489
Bearup, A. J., 155
Beck, E. M., 296
Beck, F., 261
Beck, J. V., 109
Beck, M. D., 430
Becker, D.E., 267
Becker, R., 371
Beckhorn, E. J., 220
Bedson, S. P., 487
Beebe, R. T., 263,297, 372
Beerstecher, E., 56, 59
Beglinger, E., 452
Behrens, O. K., 7, 25
Beiler, J. M., 51
Bejambes, M., 429
Bejarano, J. F. R., 299
Belady, I., 486
Beljanski, M., 169,188
Bell, E. J., 370
Bell, F.R., 285
Bell, L. G. E., 25,26
Belyavin, G., 312
Benda, L., 296
Bendigkeit, H. E., 223
Benedek, A. W., 489
Beneden, G. van, 107, 111
Benedict, R. G., 171,458
Bennett, A. M., 290, 291
Bennett, B. L., 481
Bennett, F. W., 439
Benson, A. A., 120
Bentley, R., 80
Benzer, S., 71, 196, 201, 223
Bergeim, O., 294
Berger, H., 168, 194, 219, 222
Berger, J., 452, 455
Bergersen, F. J., 39
Bergmann, E. D., 59, 170
Bergner, K. G., 449
Berk, J. E., 297
Berky, J., 167
Berliner, E., 337, 343
Berman, D. T., 188
Bernhard, K., 451
Bernhart, F. W., 63
Bernhart, G., 372
Bernhauer, K., 450, 451, 452, 462
Bernheim, F., 74
Bernheimer, A. W., 421
Bernkopf, H., 144,145
Berridge, N. J., 172
Berrie, A. M. M., 220
Berry, G. P., 371
Berry, J. W., 382
Berry, L. J., 378, 384
Berry, M. E., 252
Bertani, G., 194, 195, 203, 204, 249, 252
Bertaud, W. S., 438
Berteaux, M., 163
Bessemans, A., 137, 138,140
Beth, K., 192, 215
Bethell, F. H., 170
Bett, M., 49
Beuding, E., 353
Beumer, J., 7, 185, 200, 239
Beumer-Jochmans, M. P., 239
Beutner, E. H., 7, 10, 26, 200, 237
Bevan, E. A., 211, 224
Beveridge, J. M. R., 452, 457
Bevier, G., 299
Beyreder, J., 296
Bezer, A. E., 364
Bhat, J. V., 105, 106
Bibby, B. G., 371
Bichowski-Slomnitzki, L., 61
Bicknell, A., 116
Bicknell, A. K., 116
Bidwell, E., 421, 422
Bielig, H-J., 10, 24
Bienfet, V., 142
Bierman, H., 405
Bieser, S. M., 185
Bigham, R. S., 146
Billen, D., 16
Billet, D., 170, 220
Binkley, S. B., 48
Birch, A. J., 184
Birch-Anderson, A., 7, 26, 32, 37
Bird, O. D., 266
Bishop, J., 59
Bisset, K. A., 5, 7, 11, 12, 23, 24, 25, 26, 27, 28, 29, 31, 32, 39, 40
Bjorklund, B., 423
Bjørneboe, M., 294
Björnesjö, K. B., 373, 376
Blachère, H., 77
Black, F.L., 217
Black, L. A., 442
Black, S., 95
Blackwood, A.C., 464
Blake, F. G., 375
Blaksley, J. C., 299
Blanc, F., 160
Blanc, G., 141
Blanchard, M. C., 290, 297, 298
Bland, J. O. W., 487, 488
Blank, H., 25, 26, 27, 33, 405, 491
Blattberg, B., 382
Blindenhöfer, I., 139
Bliss, C. I., 443
Bloch, E. H., 381
Blomfield, A. M., 368
Bloom, S., 267
Bloom, W. L., 375, 376, 377, 381
Bluemle, L. W., Jr., 295
Blum, L., 368
Blumenthal, H. J., 79
Blumenthal, M. J., 295
Blunt, J. W., 290, 298
Bly, C. G., 347
Boand, A. V., 379
Boaz, H. E., 86
Bogdanov, V. M., 435
Bogert, V. V., 171
Bogorad, L., 183
Bohl, E. H., 144
Bohlander, H., 143, 144, 145
Bohnhoff, M., 65
Bohnsack, G., 171
Bohonos, N., 48, 463
Boivin, A., 378
Bolhofer, W. A., 172
Bollier, M., 294
Boltjes, T. Y. Kingma, 105, 108, 109, 111
Bolton, E.T., 80, 95
Bömeke, H., 107, 108, 111
Bond, H. W., 251
Bond, T. J., 53
Bonetti, F., 482
Boniece, W. S., 170
Bonifas, V., 195, 221
Bonner, D., 58
Boone, I. U., 56, 169
Boothe, J. H., 170
Borchers, I., 172
Bordet, J., 367
Bordowski, M., 144
Borek, B. A., 96
Borek, E., 47, 57, 251
Borensztejn, D., 296

Boresch, C., 450
Borg-Petersen, C., 138, 140, 142, 144, 145, 146
Bornemisza, G., 368
Borrel, A., 494
Bose, J. P., 285
Bostick, W. L., 280, 281
Bosworth, T. J., 413, 421
Bosworth-Brown, G., see Brown, G. B.
Boucher, R. V., 267
Bovell, C., 107, 114, 115
Bowen, H., 426
Bowie, J. H., 258, 262
Boyd, J. S. K., 248
Boyd, M. F., 153
Boyd, R. B., 88
Boyd-Orr, J., 466
Boyer, J., 142
Bozzo, A., 314
Brachet, J., 192, 215
Brackett, S., 273
Bradfield, J. R. G., 26, 32, 33, 37, 42, 134
Bradin, J. L., 280, 281
Bradley, S. G., 187
Braham, J. E., 197, 463
Brahmer, H., 461
Braley, S. A., 107, 109
Brand, T. von, 284
Brandis, H., 133-152; 133
Branoin, H. D., 265
Brannitzer, G., 217
Brans, L. M., 312, 314
Brasch, J. F., 451, 457, 465
Braude, A. I., 170, 385
Braude, R., 258, 265, 266, 267, 454, 458
Braun, W., 33, 181, 224, 378
Brawerman, G., 115
Bray, R. S., 156, 158, 159
Brede, H. D., 135
Brees, A. G., 261
Breese, S. S., Jr., 134
Bregoff, H. M., 121
Breinl, F., 336
Bresch, C., 202, 223
Brewer, C. R., 63
Bridré, J., 394
Brieger, E.M., 13, 169
Briggs, C. A. E., 429, 434
Briggs, G. M., 266, 267
Briggs, M., 443
Bringman, G., 10, 15, 24
Briody, B. A., 311, 316
Brion, G. de, 480, 481
Britten, R., 80, 95
Broadfoot, J., 400
Broadhurst, J., 492
Brockman, R. W., 173
Brockmann, H., 171, 172
Broderick, M. K., 259, 262
Brodie, A. F., 10, 76, 83
Brodribb, H. S., 291
Broers, C. M., 488
Bronstein, B., 400
Brooke, M. S., 83, 183
Brooks, B. F., 291
Brooksby, J. B., 311
Broom, J. C., 136, 140, 142, 143, 145, 146
Broquist, H. P., 48, 53, 56, 187
Bross, I. D. J., 291
Brottman, S. A., 264
Brouwer, H., 436
Brouwers, J., 142
Brown, A. H., 120
Brown, C., Jr., 263, 372
Brown, D. J., 53
Brown, G. B., 62, 63, 187
Brown, G. L., 217
Brown, G. M., 54
Brown, H. E., 146
Brown, J. H., 365
Brown, J. W., 48
Brown, R., 172, 263
Brown, R. G., 65
Brown, W., 210
Brown, W. E., 167
Brownlee, G., 167, 168, 171
Bruce, C. B., 171
Brueckner, A. L., 301
Bruff, J. A., 186, 262
Brumbach, F., 188
Brumpt, E., 133, 159
Bruner, D. W., 192
Brunetti, O., 430
Brunn, T., 172
Brunner, K. T., 143, 145
Brunner, M., 357
Brunner, R., 454
Bryant, M. P., 267
Bryan, R. W., 497
Brygoo, E. R., 146
Bryner, L. C., 109
Bryson, V., 2, 36, 40, 186, 187, 262
Brzezinsky, A., 373
Btesh, S., 144
Bubl, J. L., 452
Buchbinder, L., 442
Buchner, B., 312, 313
Bück, G., 163
Buckley, J., 399
Buckley, S., 399
Buescher, E. L., 312, 313, 315, 324
Bugher, J. C., 298, 300
Bukantz, S. C., 335, 336, 339, 340, 341, 346, 348
Bull, C. G., 374, 378
Bull, D. R., 316
Bungards, L., 295
Bunker, H. J., 451
Bunting, H., 477
Bunting, M. I., 221
Burchenal, J. H., 52, 53, 187
Burcik, E., 6
Burdon, L. K., 369
Burger, W. C., 61
Burgoon, C. F., 491
Burgwald, L. H., 440
Burke, C. P., 443
Burke, F. G., 372
Burkey, L. A., 267
Burkholder, P. R., 372
Bürki, F., 142
Burlew, J. S., 451, 465
Burlingame, P. L., 292
Burnell, J. M., 170
Burnet, F. M., 203, 224, 311, 312, 316, 318, 319, 322, 323, 324, 327, 349, 421
Burnet, McF., 318, 319
Burnett, J. H., 210
Burns, E., 295
Burns, L., 11
Burris, R. H., 92, 93, 115, 121, 459, 465
Burrows, T. W., 378
Burtenshaw, J. M. L., 373
Burton, H. S., 167
Burton, M. O., 63
Bushby, S. R. M., 171
Bushnell, O. A., 265
Busquets, A. Pumarola, see Pumarola Busquets, A.
Bussell, R. H., 294, 404, 494, 495
Buston, H. W., 59
Buswell, A. M., 108
Butler, B., 48
Butler, G., 217
Butlin, K. R., 107, 109, 111
Butorac, G., 480
Butt, H. R., 295
Buzzati-Traverso, A., 39
Buzzell, A., 251
Byatt, P. H., 480
Byrd, C. L., 432

C

Cabasso, V. J., 486
Cabelli, V. J., 186
Cabib, E., 84
Cahn, E., 221, 223
Caires, P. F. de, 299
Calhoun, K. M., 172
Califano, L., 25
Callenbach, E. W., 267
Calo, A., 7
Calvert, R. J., 294
Calvin, M., 120, 125
Cambournac, F. J. C., 160
Cameron, G., 492
Cameron, J. D. S., 258, 262
Camien, M. N., 49, 64
Campbell, A. M., 190, 199
Campbell, D. E., 353, 354, 355, 357
Campbell, D. H., 346, 354, 355, 356
Campbell, E. K., 312, 313, 324
Campbell, I. L., 429
Campbell, J. J. R., 71-104; 77, 78, 81, 83
Campbell, L., 452, 461
Campbell, L. L., 47, 56

Campbell, R. E., 294
Campbell, T. H., 167
Camps, F. E., 296, 297
Canellakis, E. S., 121
Cannon, P. R., 374, 375, 455, 465
Cantelmo, P., 203
Canter, H. Y., 168
Canti, R. G., 487
Cantino, E. C., 184, 210
Cantrell, F. P., 294
Capdupuy, J., 71
Capponi, M., 160
Capps, R. B., 290, 291, 294, 296, 297
Cardini, C. E., 84
Cardon, P. V., 296
Cariker, K., 168, 259, 262
Carlinfanti, E., 136
Carmichael, N., 396
Caron, E. L., 170, 172
Carouso, N., 186
Carpenter, K. J., 455
Carpenter, L. E., 266
Carpenter, S. J., 299
Carr, J., 398
Carr, P. H., 438
Carrel, A., 496
Carrera, G. M., 280, 282, 283
Carson, S. F., 80, 87, 88
Carter, D. B., 134
Carter, H. E., 454, 457, 458
Carvajal, F., 172
Casals, J., 366, 369
Caselli, P., 25
Caspary, E. A., 422, 423, 424, 425, 426
Cassel, W. A., 7, 25, 27, 40
Caswell, M. C., 48
Cataldi, M. S., 107
Catcheside, D. G., 463
Catlin, B. W., 219, 220
Catron, D. V., 265, 266, 267
Caughey, P. A., 215
Cavaceppi, L., 161
Cavalli, L. L., 37, 39, 40, 185, 206, 207
Cavalli-Sforza, L. L., 185, 198
Cavallo, G., 203
Cazin, J., 172, 263
Ceithaml, J., 57
Celli, A., 153
Celmer, W. D., 171, 172
Cerecedo, L. R., 452, 454
Chabbert, Y., 168
Chaigneau-Erhard, H., 169
Chain, E., 285
Chakravarty, N. K., 285
Challice, C. E., 239, 485
Chalmers, T. C., 294
Chambers, V. C., 480
Chance, C. M., 267
Chance, H. L., 7, 24, 25, 33, 38
Chang, S. L., 135, 280, 283
Chao, P. K., 189, 223
Chapman, G. B., 7, 25, 32, 37
Chardome, M., 163
Chargaff, E., 115, 209, 217
Charlebois, J., 172
Charlton, J. A., 433
Charney, J., 172
Chase, F. E., 110
Chase, M., 200, 201, 240, 242, 318
Chaudhuri, S. N., 380, 384
Cheatham, W. J., 493
Cheever, F. S., 374, 403, 493
Cheldelin, V. H., 47, 54, 55, 59, 97, 437
Chemerda, C. M., 368
Chernigoy, F., 63
Chiao, J. S., 452, 455, 460, 462
Chovnick, A., 210
Chow, B. F., 51, 267
Christensen, B. E., 59, 97
Christensen, C. M., 457, 463
Christensen, J. J., 463
Christman, J. F., 64
Christophers, S. R., 153
Chu, C. M., 312, 324, 325
Chu, E., 267
Chu, T. H., 374
Churchill, B. W., 167
Churchman, J., 59
Cia, R. A., 462
Cigarroa, J. G., 294
Ciotti, M. M., 74, 75, 169
Clapper, W. E., 168, 259, 262
Claridge, C. A., 77
Clark, A. M., 345
Clark, B. J., 208
Clark, E., 322, 323
Clark, E. C., 377
Clark, H. C., 299
Clark, J. B., 7, 25, 33, 39
Clark, L. S., 168
Clark, P. F., 364
Clark, P. J., 364
Clark, R. K., Jr., 170
Clark, R. L., 171
Clark, W. H., 430
Clarke, N. A., 249
Clarke, P. H., 421
Clausen, J. K., 64
Clawson, B. J., 365
Clayton, R. K., 123, 124
Clegg, L. F. L., 185
Cleve, E. A., 295
Cleveland, L. R., 283
Cleverdon, R. C., 25, 265, 266
Cliffton, E. E., 380
Clifton, C. E., 28, 38, 39, 63
Cline, J., 239
Clutterbuck, P. W., 451
Coates, M. E., 458
Coatney, G. R., 280
Cochrane, V. W., 79, 80
Cockburn, W. C., 296, 297
Coggeshall, L. T., 279
Cohen, G. N., 57, 61, 74, 95, 182, 183, 193, 197
Cohen, I., 296
Cohen, J. R., 264
Cohen, S. S., 79, 83, 196, 197, 201, 202, 217, 242, 244, 246, 247, 251, 261
Cohen-Bazire, G., 73, 74, 193, 197
Cohn, C., 294
Cohn, E. J., 366
Cohn, M., 57, 71, 72, 73, 74, 193, 196, 197, 422, 424, 425
Colbert, J. W., Jr., 289, 290, 293, 294, 295, 296
Coldwell, A. L., 440
Colebrook, L., 370
Coleman, N., 161
Coleman, V. R., 186, 262
Collier, R. E., 168
Collier, W. A., 143, 144, 146
Collins, E. B., 438, 439
Collins, R. J., 170
Collins, S. D., 324
Collombier, M., 144
Colmer, A. R., 106, 107, 111
Colowick, M. S., 209
Colowick, S. P., 74, 75, 169
Coman, D. R., 497
Combs, G. F., 266
Comfort, M. W., 295
Common, R. H., 465
Conn, E. C., 75
Conner, R. L., 64
Connolly, J. M., 347
Conover, L. H., 170
Conrad, H. R., 267
Conte-Marotta, R., 360
Conway, E. J., 86
Conway, E. L., 8
Cook, K. M., 365
Coons, A. H., 333-52; 337, 343, 344, 345, 346, 347, 349, 374
Cooper, C. M., 464
Cooper, H. K., 142
Cooper, W., 156, 157, 158
Coquoin, M., 393
Corbett, J. J., 51
Corelli, F., 295
Coriell, L. L., 491
Corke, C. T., 172, 173
Cormier, M. J., 92
Corradetti, A., 161
Corran, H. S., 463
Correa, M. O.A., see Alvares Correa, M. O.
Corre-Hurst, L., 142
Cosslett, V. E., 13, 169
Cottingham, E., 382
Couch, J. K., 266, 267
Coudurier, J., 163
Coulombre, J., 92
Coulston, F., 159, 161

Councilman, W. T., 282
Coursaget, J., 337
Courtenay, T. A., 245
Covell, G., 156, 157
Covert, J. M., 430
Cowie, D. B., 80, 95
Cowles, P. B., 202, 239
Cowperthwaite, J., 64
Cox, G. A., 439
Cox, H., 395, 399, 402
Cox, H. R., 486
Cox, H. W., 364
Craig, C. F., 280, 281
Craig, J. M., 293
Craig, L. C., 171
Crampton, C. F., 217, 346, 347
Crampton, E. W., 465
Cranmer, J., 292
Crawford, D., 465
Crawford, K., 167
Creech, H. J., 337, 343
Crewther, W. G., 88, 90
Crick, F. H. C., 217
Crile, D., 405
Crocker, B. F., 72
Crocker, C. G., 6
Crodel, B., 487
Cromartie, W. J., 375, 377, 381
Cros, R., 146, 160
Cruickshank, J. C., 169
Cruveilhier, L., 397
Cserey-Pechany, E., 486
Cuervo, C., 355
Cuff, P. W. W., 267
Culbertson, C. G., 170, 173
Culbertson, J. T., 285, 367
Cummings, M. M., 376
Cunha, A., 160
Cunha, T. J., 267
Cunningham, J. D., 265, 266, 267
Cunningham, K. G., 172
Curnen, E. C., 370
Cutinelli, C., 25, 119, 121
Czulak, J., 434, 435, 437, 438, 439

D

Dacre, J. C., 441
Dagley, S., 80, 97
Dale, R. F., 464
d'Alessandro, G., 160
Dalling, T., 411, 415, 421
Daly, J. M., 463
Dam, R. van, 140
Damm, H., 457
Dammin, G. J., 335, 336, 339, 340, 341, 346, 348
D'Amore, A., 7
D'Amore, K., 144
Daniel, L. J., 51
Daniel, P. M., 278, 284
Daniels, J. B., 374
Dankwerts, P. W., 462, 465
Darken, M. A., 458
Dass, C. M. S., 215
Daugherty, P. M., 465
Davenport, H. E., 125
Davey, P., 170
Davey, V. F., 167
Davidson, A. G., 364
Davies, E., 433
Davies, M., 395, 399
Davies, R., 181
Davies, W. L., 364
Davis, B. D., 57, 59, 92, 169, 182, 183, 185, 187, 196
Davis, D., 109
Davis, D. E., 142
Davis, G. E., 133
Davis, J. C., 10, 11, 13, 15
Davis, N., 294
Davis, P. A., 441
Davis, R. L., 51, 267
Davis, S., 267
Dawes, E. A., 80
Dawson, I. M., 7, 17, 240
De U. N., 285
Dean, A. C. R., 185
Dean, F. M., 172
Dean, R. F. A., 465
Deane, D. D., 438, 439
Deane, H. W., 344, 349
Deane, N., 294
Dearing, W. H., 257, 262, 263, 264, 265, 372
DeBoer, C., 172
DeBusk, A. G., 97
DeBusk, B. G., 86
Decaris, M. L.-J., 172
DeCarlo, M. R., 249
Décourt, P., 160
DeDios Calle, J., 197, 463
DeFiebre, C. W., 79
Deiches, H., 187
Deichmiller, M. P., 337, 346, 348
Delage, B., 141
De la Haba, G., 83
Delamater, E. D., 23-46; 1, 2, 10, 23, 24, 25, 26, 27, 28, 31, 32, 33, 35, 36, 37, 38, 39, 40
DeLapi, M. M., 349
Delaporte, B., 32
Delaunay, A., 378
Delbrück, M., 202, 249, 251
Delchamps, E. W., 109
Delluva, A. M., 95
Delmotte, P., 172
Delmotte-Plaquee, J., 172
Delp, M. H., 294
DeLustig, E., 403
Delwiche, E. A., 80, 87
DeMars, R. I., 201, 223, 241, 242
Demerec, M., 221, 223
Demmler, G., 452, 460
DeMoss, R. D., 80
Dempster, G., 312, 313
Denber, H. C. B., 293
Denk, V., 109
Denman, R. F., 115
Dent, C. E., 294
DePace, N., 404, 405
DeRenzo, E. C., 49
De Robertis, E., 6
Derom, R., 137, 138
De Rose, A. F., 171
De Salles Gomes, L., 142
Deschiens, R., 280
DeSerres, F. J., 221
DeTurk, W. E., 74
Devidé, Z., 106
Devlin, K. A., 172
Dewey, D. L., 115, 182, 195
Dewey, V. C., 51, 52
Diamond, L., 401
Dianzani, M. U., 209
Dick, E. C., 265, 266
Dick, G. W. A., 290, 299, 300
Dickerman, G. K., 454
Dickos, J., 403
Diefenbach, W. C. L., 296 297
Diemair, W., 450, 452, 462
Dienes, L., 206
Dietrich, L. S., 54
Dillon, E., 442
DiLorenzo, J. C., 264, 265
Di Marco, A., 169
Dintzis, R. Z., 170
Dirr, K., 451
Dishburger, H. J., 92
Ditlevsen, E., 224
Diverneau, G., 193, 200
Dixon, F. J., 335, 336, 337, 339, 340, 341, 342, 346, 348
Dixon, J., 200, 201, 242
Doan, F. J., 440
Dod, K., 405
Doermann, A. H., 202, 241
Doery, H. M., 173
Doflein, F., 133
Dolby, R. M., 429
Dold, H., 370
Dole, V. P., 276, 374
Dolin, M., 85
Dolin, M. I., 76
Doljanski, L., 497
Doll, E. R., 485
Domenici, T. J., 293
Dommelen, C. K. V. van, 294
Donaldson, D. M., 368
Donatien, A., 141, 145
Dondero, N. C., 206
Donikian, M., 261
Donker, H. J. L., 113
Donovan, F. W., 184
Donovick, R., 169, 171
Dorfman, A., 56, 375
Doria-Medina, J., 299
Dornbush, A. C., 170
Dorner, R. W., 217
Dorrestein, R., 120

Doudney, C. O., 58, 183
Doudoroff, M., 78
Doughty, C. C., 96
Douglas, H. C., 117
Doull, D. P., 438
Dowling, H. F., 259, 261, 262
Downey, M., 8
Downie, A. W., 484
Downs, W. G., 161
Dozois, T. F., 366, 368
Drake, M., 293, 297
Drake, M. E., 290, 291, 296, 297, 298
Draper, C. C., 162
Drews, B., 463
Drinnon, V. P., 159, 160
Driscoll, C. A., 62
Dubert, J. M., 337
Dubey, G. A., 450, 452, 462
Dubin, I. N., 159, 160
Dubos, R., 13
Dubos, R. J., 376, 377, 380
Ducci, H., 292, 294, 295
Duchow, E., 117
Dudgeon, J. A., 312, 326
Duffy, C. E., 364
Duguid, J. P., 2, 3, 23
Duke, B. O. L., 162
Dukes, A. M., 216, 259
Dukes, C. D., 216, 259
Dulbecco, R., 173, 203, 223, 320, 321, 478, 482
Dulmage, H. T., 171
Duncan, C. W., 267
Duncan, D., 477
Dunn, C. G., 449, 451, 452, 457
Dunn, M. S., 49, 64, 135
Duraiswami, S., 211
Duran-Reynals, F., 173, 377
Duran-Reynals, M. L., 377
Dusi, H., 188
Dutcher, J. D., 171
Duthie, E. S., 285
Duysens, L. N. M., 122, 124, 125, 126
Dvonch, W., 171
Dye, W. E., 261
Dymsza, H., 267

E

Eades, C. H., Jr., 49
Eagle, H., 64, 187, 221
Eagles, B. A., 81
Eakin, R. E., 56
Earle, W. R., 497
Early, L., 263, 372
Eaton, M. D., 493
Ebel, J. P., 14, 15
Ebeling, A. H., 496
Ecker, E. E., 366, 368, 379
Eckerling, B., 373
Eckhardt, R. D., 294
Eddy, A. A., 195, 220, 221
Edeson, J. F. B., 155
Edlinger, E., 239
Edney, M., 318, 319
Edward, D. G., 206
Edwards, J., 248
Edwards, P. R., 193, 208
Egami, F., 92
Eggleston, L. V., 80
Eguchi, S., 453, 460
Ehrensvärd, G., 59, 119, 121, 452, 453
Ehret, C. F., 219
Ehrhard, H.-B., 28, 38, 39
Ehrlich, R., 443
Eichman, H. L., 293, 294
Eichman, P. L., 290, 294, 298
Eidam, C. R., 476, 477
Eigelsbach, H. T., 224
Eisen, H. N., 334
Eisenberg, G. M., 264
Eisenberg, M. A., 80, 119
Eisenman, E., 172
Eisler, D. M., 379
Eisner, H. J., 170
Eklund, H. W., 424
Ekstrom, D., 452, 454
Elam, J. F., 266
Elberg, S. S., 380
Elek, S. D., 423
Elford, W. J., 7, 239, 240
Elghammer, R. M., 298
Elion, G. B., 62, 187
Eliot, T. S., 274
Elliker, P. R., 185, 436, 438, 439, 440
Elliot, W. H., 57
Ellis, B., 170
Ellison, D., 433
Elsden, S. R., 119, 125
Elton, N. W., 299
Elvehjem, C. A., 54, 265, 267, 364
Emerson, G. A., 65
Emerson, K., 276
Emerson, R., 465
Emmons, C. W., 372
Enders, J. F., 473-502; 368, 473, 476, 477, 480, 482, 483, 484, 488, 489, 492, 493
Endicott, F. C., 334, 335, 338
Enebo, L., 449, 450, 452
Enerson, D. M., 298
Engel, H., 106
Engelhardt, E. L., 62
Engelmann, T. W., 116, 123
England, B., 301
Englesberg, E., 80
English, A. R., 170, 171
Enns, T., 337
Enright, J. B., 430
Entner, N., 77, 78
Ephrussi, B., 181, 485
Epstein, H. T., 219, 237
Epstein, J., 401
Erdos, E., 382
Erf, L., 405
Erickson, J. O., 346
Errebo-Knudsen, E. O., 145
Erwin, C. P., 257, 264
Erwin, R. F., 365
Esplin, D. W., 382
Esser, K., 192, 212
Esseveld, H., 146
Ettinger, R. H., 290, 291
Evenhuis, N., 436
Evans, A. M., 163
Evans, A. S., 292, 293, 295
Evans, C. A., 478, 480
Evans, D. G., 421
Evans, E. A., Jr., 237-56; 200, 237, 243, 244, 245, 246
Evans, H. J., 92
Evenhuis, N., 436
Evenson, A., 265
Ewing, F. M., 161

F

Fabel, K., 453, 460
Fabian, F. W., 443
Fabricant, C., 7
Fabioli, O., 170
Fagraeus, A., 140
Fahmy, M., 463
Fairley, N. H., 153, 156, 157, 276
Fairlie, C. W., 262, 263, 372
Fanelli, G. M., 171
Farmer, F. H., 465
Farquhar, J. A., 291, 295
Farquhar, J. D., 290, 291, 295, 298
Farrant, J. L., 7, 239
Fastier, L. B., 479
Faulkner, G. H., 497
Fauré-Fremiet, E., 106
Faust, E. C., 280, 283
Faust, R. A., 463
Fazekas de St. Groth, S., 370
Feeley, N., 298
Feemster, R. F., 483
Fehrsen, P., 364
Feigelman, H., 116
Feinberg, J. G., 355
Feldman, H. A., 275
Feldman, W. H., 261
Felix, A., 194, 205, 239
Fell, R. V., 267
Feller, A. E., 488, 489
Feltes, J., 385
Felton, E. A., 53
Felton, L. D., 349, 381
Fenn, W. O., 382
Fennell, D. I., 214, 463
Fenner, F., 349
Fenton, E. L., 422, 423, 424, 425, 426
Ferebee, S. H., 261
Ferguson, L. C., 144
Ferlauto, R. J., 171
Fernandez, B., 181, 223, 252
Fernstrom, G. A., 464
Ferris, A. A., 144
Fertig, H., 479
Few, A. V., 9, 171

Fewster, M., 97
Ficq, A., 347
Field, H. J., 141
Field, J. W., 155
Field, M. F., 171
Fields, M., 347
Fieldsteel, A. H., 384
Fifield, C. W., 443
Fildes, P., 199
Filfus, J., 50
Fincham, J. R. S., 96
Findlay, G. M., 280, 400, 403
Fine, J., 334, 337
Fink, H., 449, 452, 456
Fink, R. M., 337
Finkelstein, M. H., 336
Finland, M., 168, 170, 188, 259, 262, 364
Finlay, A., 171
Fiore, J. V., 451, 458
Fischel, E. E., 364
Fischer, A., 489, 497
Fischer, R., 9
Fisher, H. F., 75
Fisher, H. W., 201, 241, 242
Fisher, W. P., 172
Fitch, D. R., 297
Fitzgerald, P. L., 209
Fitz-James, P. C., 41
Fitts, R. A., 171
Flavin, M., 63
Flewett, T. H., 23, 485
Flickinger, M. H., 463
Fling, M., 196, 197
Flippin, H. F., 264
Flodin, N. W., 455, 457
Florentino, G. L., 476, 477, 482
Florey, H. W., 167
Florman, A. L., 488
Flynn, E. H., 53
Foard, M., 367
Foda, O., 79
Fogg, G. E., 465
Foley, E. J., 364
Foley, G. E., 52, 186
Folkers, K., 55, 173
Follette, J. H., 383
Fonseca, F. da, 160, 163
Foote, M. W., 172
Forbes, B. R. V., 144
Forbes, E., 213
Forbes, M., 267
Ford, J. C., 291
Ford, J. E., 50
Ford, J. H., 170
Formal, S. B., 208
Forrest, I. S., 169
Foster, E. M., 437, 441
Foster, H. G., Jr., 431
Foster, J. W., 54, 65, 88, 118, 168, 449, 463
Foster, P. D., 296
Foster, R. A. C., 241
Fouace, J., 216
Foulkes, E. C., 80, 81
Fountaine, F. C., 267
Fouts, J. R., 169
Foy, H., 274
Fowden, L., 453
Fowell, R. R., 463
Fox, A. S., 210
Fox, J. P., 496
Fox, L., 364
Fox, R. A., 298
Franchi, C. M., 6
Francis, 370
Francis, G. E., 346
Francis, T., Jr., 311, 312, 314, 315, 323, 325, 326, 327, 383
Francke, C., 294
Frank, A. J., 172
Franke, K., 296
Frankel, J. W., 301, 302
Franklin, A. L., 49
Franklin, K. J., 278, 284
Franklin, M., 294
Franklin, R. E., 217
Franklin, R. M., 219
Franks, M. B., 359
Franz, I. D., 116
Fraser, D., 121, 199, 203, 217, 218, 237, 238, 241
Fraser, D. E., 296
Fraser, K. B., 203, 318, 319
Frazier, W. C., 461, 464
Fred, E. B., 458
Fredericq, P., 185, 196, 205, 252
Freed, M., 375, 377
Freeman, G. G., 172
Freeman, V. J., 252
French, C. S., 120, 122
French, R. C., 240, 241, 242, 243
Frenkel, A. W., 120
Frenkel, H. S., 479
Friedewald, W., 399
Friedman, M., 202, 219, 239
Friedman, M. E., 441
Friedman, S., 201, 247
Friedrich-Freksa, H., 218
Fries, N., 193, 215, 224
Friesen, A. M., 379
Fritze, E., 382
Frocheschi, C., 354, 355
Frunder, M. A., 135
Fruton, J.S., 59, 60, 453
Frye, W.W., 280
Fryer, H.C., 267
Fryth, P.W., 172
Führer, F., 140, 147
Fujishige, H., 112
Fukumi, H., 6
Fulde, R.C., 443
Fulthorpe, A.J., 423
Fulton, F., 322
Fulton, J.D., 276, 284
Fusillo, M.H., 169, 170, 171, 261

G

Gabuzda, G.J., 294
Gädeke, R., 147
Gaden, E.L., 185, 219, 464
Gaethgens, W., 140
Gaffron, H., 118
Gaither, N., 219, 222
Gajdusek, D.C., 477
Gale, E.F., 57, 167, 181
Gale, G.R., 91
Galesloot, T.E., 434, 437
Galindo, P., 299
Gall, D., 278
Gallais, P., 160
Gallop, R.C., 382
Galloway, I.A., 311
Galluzzi, N.J., 298
Galvis, A.G., see Gast Galvis, A.
Galyen, L.I., 25
Gambescia, J.M., 294, 295
Gandelman, B., 115, 217
Garber, E.D., 183, 186
Garcia, E., 299
Garcia, E.Y., 160
Gard, D.I., 267
Gardner, D.L., 262, 263
Gardner, G.M., 172
Gardner, H.T., 292, 293, 294
Gardocki, J.F., 171
Garen, A., 201, 239
Garlick, C.H., 144
Garlock, J.H., 371
Garner, H.R., 463
Garnham, P.C.C., 153-66; 153, 154, 155, 156, 157, 158, 159, 160, 162
Garnjobst, L., 210, 214, 463
Garvey, J.S., 346
Gastel, R., 83
Gast Galvis, A., 299
Gattani, M.L., 167, 463
Gauld, R.L., 292, 313
Gavard, R., 78
Gavosto, F., 347
Gaylin, S.G., 314
Gayot, G., 141, 145
Geckler, R.P., 189, 219
Gee, F., 299
Gee, L.L., 266
Gehrig, R.F., 96
Geiman, Q.M., 136
Gelderen, H. van, 118, 120
Gell, P.G.H., 382
Gellis, S., 293
Gellis, S.S., 289, 291, 294, 296, 297
Geoghegan, H., 294
Georgi, C.E., 11
Gerke, J.R., 108
Germer, W.D., 367
Germuth, F.G., Jr., 337
Gerstley, L., 3

Gertani, G., 248, 249
Gest, H., 88, 89, 114, 115, 116, 118, 120, 121
Gey, G. O., 403, 404, 476, 478, 479, 481, 482, 485, 487, 491, 493
Gey, M. K., 481
Giantini, J., 239
Gibbs, M., 84
Gibor, A., 80
Gibson, D. L., 452, 460, 461
Giesberger, G., 117
Giges, B., 295
Gildemeister, E., 490
Giles, N. H., 183, 198, 221
Gillen, D. H., 246
Gilles, H. M., 283, 284
Gillespie, D. G., 80
Gilmore, L. K., 476, 484
Ginder, D., 399
Ginsberg, D., 374
Ginsberg, H., 402
Ginsberg, H. S., 317, 367
Giovanella, R., 144
Gispen, R., 137
Gitlin, D., 335, 343, 344
Gladstone, G. P., 412
Glauert, A. M., 13, 169
Gledhill, A. W., 290, 312, 313, 315, 324, 325
Gleen, H., 109, 110, 112
Glenny, A. T., 337, 338, 411, 414, 415, 421, 422
Gloor, E. T., see Tarr Gloor, E.
Glotowa, E. W., 421
Glover, J., 118, 120
Glover, R. E., 312, 326, 413
Gochenour, W. S., 144
Gochenour, W. S., Jr., 134, 135, 140, 144, 146
Godman, G. C., 374, 477
Godzesky, C., 167
Goebel, W. F., 7, 185, 200, 240
Goedheer, J. C., 124
Gold, J. J., 364
Goldberg, R., 63
Goldblatt, H., 295, 452, 456
Goldbloom, R. S., 295, 296
Goldblum, N., 154
Goldman, M. L., 442
Goldschmidt, R., 33
Goldstein, L., 442
Gonshery, L., 168
Goodloe, S., 375
Goodlow, R. J., 378
Goodman, M. B., 298
Goodwin, M. H., Jr., 162
Goodwin, T. W., 122, 123, 458
Gorbach, I. J., 483
Gorcica, H. J., 454, 458
Gordon, H. A., 365
Gordon, H. L., 51
Gordon, J., 368
Gordon, J. T., 62
Gordon, M., 167
Goreczky, L., 368
Gorman, W. F., 293
Gorovitz, H., 143
Gosling, R. G., 217
Gotlieb, T., 144, 203, 319, 320
Goto, T., 487
Gottlieb, D., 170
Gots, J. J., 247, 251
Gots, J. S., 201, 205
Goudie, J. G., 142
Gould, I. A., 440
Gould, J. C., 258, 262
Gowen, J. W., 183
Grabow, J., 49
Grace, J. B., 5
Grady, R. P., 111
Graff, S., 63
Graham, A. F., 200, 201, 237, 240, 241, 242, 243
Graham, D. M., 438, 439
Graham, V. E., 452, 460, 461
Gram, H. G., 135, 147
Granick, S., 183, 215
Grant, G. A., 458
Gratia, A., 368
Gray, D. H., 39
Gray, I., 167
Gray, J. D., 263
Gray, L. F., 51
Gray, N. M., 95
Greco, F. del, 295
Green, M., 93, 115, 184
Green, P., 294
Green, R., 383
Greenberg, J., 280
Greene, H. C., 458
Greene, M. R., 135
Greene, V. W., 440
Greenlee, R. G., 298
Greenspan, E. M., 294
Greenstein, N. M., 296, 297
Greenwald, L., 296
Gregory, M. E., 450
Greiner, D. J., 296
Grell, K. G., 216
Grenan, M. M., 168
Griffiths, D. G., 433
Griffiths, L. A., 458
Grillo, J., 137
Grogg, E., 383
Groman, N. B., 185, 205, 252
Gröne, H., 171
Gros, F., 167
Gros, P., 337
Grosche, C. A., 432
Grossberg, D. B., 424
Grosser, A., 452, 462
Grossman, B. J., 298
Grossman, J., 109
Groupe, V., 173
Grove, J. F., 172
Grunberg, E., 262
Grunberg-Manago, M., 80
Grundy, W. E., 171
Gsell, O., 133, 142, 143, 144, 145
Guelin, A. M., 239
Guest, C. M., 263, 372
Guex-Holzer, S., 2, 3
Guggenheim, K., 267
Guida, V. O., 142
Gunn, F. D., 374
Gunnison, J. B., 168, 186, 262
Gunsalus, C. F., 16
Gunsalus, I. C., 16, 78, 80, 85, 86, 91
Gunter, S. E., 91
Gupta, P. C. Sen, see Sen Gupta, P. C.
Gurevitch, J., 373
Gurin, S., 80
Gutierrez, J., 266
Guzman-Garcia, J., 265, 266
György, P., 267, 295, 452, 456

H

Haagen, E., 487, 488, 489, 490, 496
Haam, E. von, 405
Haardick, H., 10, 24
Haas, F. L., 168, 181, 219, 222, 223, 252
Haas, G. J., 171
Haas, V. H., 161
Haber, P., 393
Hackett, A. J., 183
Haddard, S. A., 211
Haddow, A. J., 154, 299
Hadfield, A. S., 171
Haefele, J. W., 374
Haehn, H., 449
Haenseler, C. M., 172
Haeseler, G., 449
Haga, K., 112
Hager, L. P., 86
Haguenau, J., 397
Hahn, E., 209
Hahn, F. E., 10
Hahn, R. G., 300
Haight, T. H., 170, 259
Hajny, G. J., 452, 455
Halberstaedter, L., 497
Halbert, S. P., 372
Hale, C. M. F., 5, 6, 7, 27, 31
Hale, F., 48
Halevi, C., 144, 145
Halevy, S., 267
Hall, A. G., 120
Hall, H. H., 458
Hall, J. W., 296
Hall, L., 295
Hallauer, C., 136, 403, 485
Hallmark, G. D., 92
Halse, B., 429
Halvorson, H. O., 72, 73, 196, 432
Ham, W. E., 267
Hamada, K., 172
Hamburger, C., 294
Hamilton, L., 63
Hamilton, P. B., 93
Hämmerling, J., 215
Hammerman, D. J., 295

Hammon, W. McD., 311
Hammond, L. A., 434
Hamolsky, M. W., 296
Hampil, B., 298
Hamre, D. M., 171
Hanan, R., 364
Handler, B. J., 367
Hanes, M. E., 65, 171
Hankin, L., 432
Hannan, M. L., 452, 461
Hannay, C. L., 40, 185, 205
Hansen, R. G , 84
Hansen, S. B., 170
Hanson, R. J., 379
Happold, F. C., 97
Hara, K., 290, 298
Harder, R., 193
Harfenist, E. J., 171
Harford, C. G., 364
Harinasuta, C., 280, 283, 284
Harkness, W. L., 438
Harm, W., 194, 220, 221
Harmsen, G. W., 109
Harper, A. E., 267
Harrington, J. A., 296, 297
Harris, E. E., 452, 455, 461
Harris, H., 380
Harris, S., 290, 298
Harris, S. A., 55
Harris, T. N., 290, 298
Harrison, A., 79
Harrison, J. W. E., 167
Harrison, R., 403
Harrison, R. W., 495
Harris-Smith, P. W., 382
Hart, E. B., 265
Hartelius, V., 224
Hartman, P. E., 2, 7, 10, 11, 25, 39, 200, 237
Hartman, R. S., 224
Hartmann, F. W , 298
Hartmann, I., 267
Hartmann, M., 216
Hartmans, S., 429
Hartsook, E. W., 267
Haskins, F. A., 81, 184, 189, 196
Hassinger-Huizinga, H., 215
Hastings, A. B., 170
Hastings, J. W., 92
Hatano, S., 290, 298
Hatch, T., 365
Haurowitz, F., 336, 346, 347
Hauser, M. M., 265, 459
Hausmann, W., 171
Havens, W. P., Jr., 289-310; 289, 290, 291, 292, 293, 294, 295, 298
Hawking, F., 156, 160
Hawkins, J. D., 346
Hawkinson, V., 294
Hawn, C. V., 341
Hawthorne, D. C., 211
Haxo, F., 122
Hayaishi, O., 88, 97
Hayes, J. E., Jr., 77
Hayes, W., 37, 39, 40, 195. 206, 207, 208, 252
Hazen, E. L., 172, 263
Heald, P. J., 460
Healey, M., 426
Heath, E. C., 79
Heatley, N. G., 167
Hecht, L., 14
Hecke, F., 479
Heckly, R., 381
Hedvall, J., 6, 8
Heenan, E. F., 51
Hegarty, C. P., 463
Hegner, R., 160, 280
Hegner, R. W., 273, 276
Hehre, E. J., 85
Heidelberger, M., 337, 342, 349, 366, 369
Heilman, F. R., 145, 167, 262, 263
Heines, V., 452, 461
Heinrich, E., 449
Heinrich, M. R., 52
Heisch, R. B., 155
Heissler, I., 295
Heitmiller, R. F., 86
Hellman, N. N., 85
Hench, M. E., 369
Henderson, H. J., 10
Henderson, W. M., 311
Hendlin, D., 47-70; 48, 50, 51, 53
Heneage, P., 94
Hengy, F. C., 246
Henle, G., 290, 296, 297, 298, 322
Henle, W., 290, 296, 297, 298, 312, 320, 321, 322, 326, 364
Henley, D. A., 377
Henley, E. J., 185, 219
Henneberg, G., 168
Hennensen, W. A., 314
Henry-Eveno, J., 393
Henssler, W., 453
Heotis, J., 11
Herbert, D., 413
Hermann, E. C., Jr., 186
Herrera, J. M., 299
Herreweghe, E., 134
Herring, R., 224
Herriott, R. M., 237, 247
Hershey, A. D., 200, 202, 217, 240, 242, 246, 318
Hervey, A., 50, 188
Herzberg, K., 203
Hess, E. L., 344
Hesseltine, C. W., 48
Heubschman, C., 25, 26
Heuser, G. F., 265
Heusser, H., 144
Hewitt, L. F., 12, 185, 205
Hewitt, L. K., 252
Heyl, D., 55, 173
Heyningen, W. E. van, 411, 412, 421, 422
Heytler, P., 49
Hibbs, J. W., 267
Hickey, R. J., 64, 65
Hidalgo, L., 452, 462
Hiernaux-L'Hoest, D., 163
Hiernaux-L'Hoest, J., 163
Higgens, C. E., 170
Higginbottom, C., 434
Higgins, G., 405
Highman, B., 280
Hightower, J. A., 135
Higuchi, K., 63
Hilditch, T. P., 451
Hill, A. G. S., 344, 349
Hill, C. H., 86, 266.
Hill, D. C., 265
Hill, E., 293
Hill, G. A., 382
Hill, M. B., 202
Hill, R., 125
Hill, T. S., 275, 279, 285
Hilleman, M. R., 311-32; 311, 312, 313, 315, 324, 476, 484
Hillier, J., 7, 8, 10, 25, 26, 32, 37, 200, 237
Hills, G. M., 47
Hilz, H., 90, 94
Himsworth, H. P., 282, 456
Hinckle, M. E., 107
Hinde, I. T., 382
Hinman, F., Jr., 264
Hinman, J. W., 170, 172
Hinshelwood, C., 185, 195, 221
Hirsch, A., 429, 435
Hirsch, H. E., 120
Hirsch, H. M., 81, 184, 190
Hirsch, J., 169
Hirsch, J. G., 169, 376, 377
Hirsch, M. L., 61, 95, 182, 183
Hirsch, M. M., 259
Hirst, G. K., 203, 311, 312, 314, 317, 318, 319, 320
Hiscox, E. R., 429
Hiss, P. H., 368
Hitchings, G. H., 62, 187
Hixson, A. W., 464
Hoag, W. G., 140
Hobby, G. L., 167, 168, 170, 186, 261, 262
Hoberman, H. D., 48
Hobson, A. C. S., 296
Hoch, H., 294
Hochester, R. M., 82
Hochstein, F. A., 171
Hock, A., 456
Hodes, H. P., 432
Hoehn, M. M., 172, 173
Hoerburger, W., 449
Hoffman, C. A., 85
Hoffman, H. J., 23, 28, 39
Hoffmann, C. E., 49
Hofman, T., 92, 113, 115
Hofsten, B. von, 224
Holderby, J. M., 461
Hollaender, A., 219, 220, 298
Holland, B. C., 379

Holland, J. F., 295
Hollander, F., 371
Hollands, S. J., 107, 109, 111
Holmes, R., 72
Holton, C. S., 210
Hood, E. G., 429
Hoogerbeide, J. C., 458
Hoogstraal, H., 153
Hoover, S. R., 458, 461
Hopkins, B. E., 337
Hopper, J., Jr., 264
Horecker, B. L., 82
Horgan, E. S., 300
Horii, K., 462
Hornberger, C. S., Jr., 86
Horne, R. W., 7, 8
Horowitz, N. H., 57, 196, 197, 198
Horsfall, F. L., Jr., 173, 311, 312, 314, 317, 318, 326, 367
Horstmann, D. M., 292
Horton, R., 261
Horváth, S., 3
Hosansky, N., 171
Hosler, P., 167
Hosoya, S., 172
Hospodka, J., 451
Hoster, H., 405
Hotchin, J. E., 7, 239, 240
Hotchkiss, R. D., 167
Hotta, S., 290, 298, 301, 302
Hottle, G. A., 421, 424
Hounie, E., 453, 460
House, F., 455
Housewright, R. D., 93
Houtermans, T., 221
Houwink, A. L., 5, 8
Howard, J. G., 413, 414
Howell, A., 170
Howell, D. S., 297
Howie, J. W., 364
Howitt, B. F., 367
Hoyle, L., 321, 368
Hsia, D. Y., 289, 291, 294
Huang, C. H., 477, 495
Huan-Ying Li, 142
Hubbell, E. J., 477
Hubbert, M., 263
Huber, L., 11
Hudack, S. S., 489
Hudgins, P. C., 376
Huebner, R. J., 430, 476, 484
Huebschman, C., 214
Huerga, J. de la, 294
Huff, C. G., 153, 154, 158, 159, 160, 161, 162
Huff, J. W., 62
Huffman, C. F., 267
Huggins, M. L., 6
Hughes, L. P., 450, 452, 461, 462
Hughes, W. H., 206
Huhtanen, C. N., 50
Human, M. L., 246, 252
Humfeld, H., 462
Humm, F. D., 347
Humphreys, J., 53
Hungate, R. E., 462, 464
Hunt, G. R., Jr., 205, 251
Hunter, G. J. E., 83, 439
Hunter, M. E., 2, 26, 28, 31, 32, 33, 36, 40
Hunter, P. F., 58
Huppert, M., 172
Hussong, R. V., 443
Hutchings, B. L., 48, 49, 172, 265
Hutchison, D. J., 52, 53, 187
Hutner, S. H., 49, 50, 64, 117
Hutton, W. E., 105, 113
Huybers, K., 205, 251
Hyatt, M. T., 210
Hyde, R. R., 489, 490, 492
Hyman, S., 296
Hyndman, L. A., 93, 115

I

Ichihara, K., 91
Ides, E., 171
Igeta, H., 458
Ilavsky, J., 364
Illa, R., 141
Ingraham, J. S., 333, 336, 346
Inoki, S., 160
Inskeep, G. C., 461
Ionesco, H., 185, 205
Ipsen, I., Jr., 291
Isaacs, A., 312, 313, 315, 324, 325, 326
Ischii, N., 487
Isenberg, H. D., 114
Israeli, C., 488
Iterson, W. van, 3, 5
Ivánovics, G., 3, 486, 490,492
Iwata, Y., 452, 462

J

Jackson, E., 298
Jackson, G. G., 259, 261, 262
Jackson, R. W., 85, 458
Jackson, W. G., 172
Jacob, F., 72, 185, 201, 203, 204, 205, 223, 224, 249, 250, 251
Jacobs, M. G., 451
Jacobs, R. L., 266
Jacobs, S. E., 185
Jacobson, W. E., 189
Jacox, R. F., 367
Jacusiel, F., 144
Jaffe, H., 183
Jakob, A., 134
James, A. P., 211
James, C. G., 171
James, G., 298
Janeway, C. A., 289, 295, 296, 335, 341, 344
Janowitz, H. D., 371
Janssen, E. F., 348
Jasewicz, L., 452, 460
Jaswant Singh, see Singh, J.
Jawetz, E., 168, 186, 262, 264
Jefferson, W. E., 88
Jeffrey, G. M., 157
Jellison, W. L., 430
Jennings, C. G., Jr., 296
Jennings, J. C., 172
Jenny, J., 143
Jensen, A. H., 265, 266
Jensen, H. L., 13, 111, 113
Jensen, J., 183, 198
Jensen, K. E., 314
Jermyn, M. A., 85
Jerne, N. K., 414
Jerrel, E. A., 218, 237
Jersild, M., 294
Jesaitis, M. A., 7, 185, 200, 240
Jezeski, J. J., 429-48; 440, 442
Jinks, J. L., 211, 221
Johansson, K. R., 265, 266
Johns, R. G. S., 294
Johnson, A., 370
Johnson, B. A., 262
Johnson, B. C., 267, 458
Johnson, C. C. R., 477
Johnson, C. M., 280
Johnson, D. W., 146
Johnson, F. A., 39
Johnson, G. C., 167
Johnson, L., 261
Johnson, L. E., 172
Johnson, M. C., 452, 455
Johnson, M. J., 167, 455, 461, 464
Johnston, R. V., 144
Johnstone, D. B., 172
Johnstone, H. G., 280, 281
Joklik, W. K., 115, 239, 247
Jolit, M., 197
Jones, D. B., 465
Jones, E. R , 285
Jones, E. S., 274, 278, 283
Jones, L. W., 63
Jones, M. E., 90, 94
Jones, M. J., 56
Jones, R. G., 433
Jones, R. N., 337, 343
Jong, L. E. den dooren de, 249
Jordan, J., 290, 298
Jordan, R. T., 364
Jordão, F. Magaldi, see Magaldi Jordão, F.
Jordon, W. S., Jr., 314
Josephson, D. V., 440
Josephson, E. S., 280
Joshua, J. O., 142
Josselyn, L. E., 170
Jotz, A. C., 312, 313, 315
Joyner, L. P., 284
Jude, A., 193, 200
Jukes, T. H., 49, 267, 458
Julianelle, L., 403
Junge, J. M., 76
Juni, E., 14

K

Kabat, E. A., 364
Kaekenbeeck, A., 142
Kahler, H., 217
Kakihara, Y., 91
Kalan, E., 57
Kaleita, E., 49
Kalich, J., 143
Kalkstein, M., 296
Kallio, R. E., 80, 194
Kalmansohn, R. W., 295
Kambar, C. S., 443
Kamen, M. D., 14, 109, 118, 119, 120, 121, 125
Kamitsuka, P., 481
Kane, C. A., 296
Kanter, U., 143, 144
Kapfhammer, J., 453
Kaplan, A. S., 195, 203, 204, 431
Kaplan, M. H., 337, 343, 344, 345, 349
Kaplan, N. O., 74, 75, 81, 169, 184
Kaplan, R. W., 219
Karibian, D., 83
Kark, R. M., 294
Karlson, A. G., 261
Karlsson, J. L., 109
Karow, E. O., 464
Karzon, D. T., 367, 494
Kashiwagi, Y., 290, 298
Kass, E. H., 364, 474
Kästli, P., 429
Katagiri, M., 183
Katchalski, E., 61
Kathe, J., 143, 144
Kato, N., 56
Katz, E., 120, 122
Katz, J., 93, 94, 95
Katz, R., 295
Katznelson, H., 79, 93
Kaudewitz, F., 218
Kaufmann, G. G., 291
Kausche, G. A., 10, 24, 401
Kato, N., 169
Kawaguchi, S., 120
Kazenko, A., 85
Kearney, E. B., 97
Kegeles, G., 424
Kehar, N. D., 275
Keigler, M. N., 25
Keilin, D., 82
Keipper, C. H., 463
Keitt, G. W., 172
Kellenberger, E., 10, 11, 23, 24, 25, 26, 27, 37
Keller, T. C., 292
Kelly, J. W., 266
Kelly, K., 405
Kelly, M. C., 370
Kempe, C. H., 489
Kempf, A. H., 364
Kempf, J. E., 379
Kendall, F. E., 342
Kendall, R. E., 262, 263, 372
Kennell, J. H., 296
Kenny, G. C., 144
Keosian, J., 222
Kerby, G. P., 379
Kerin, L., 115
Kermack, W. O., 111
Kern, R. A., 7, 25
Kern, S. F., 25, 86
Kerr, J. A., 298
Kerr, S. E., 63
Kessel, J. F., 280
Kester, L. T., 442
Keston, A. S., 334
Ketsu, B., 452, 462
Kibrick, S., 482
Kidd, J. G., 367
Kidder, G. W., 51, 52
Kiem, I., 473
Kihara, H., 60
Kilbourne, E. D., 314
Kile, R. L., 374
Kilham, L., 494
Kimball, C. P., 337
Kimball, R. F., 189, 219, 222
Kimura, R., 290, 298, 301, 302
King, D., 109
King, H., 375
King, J. O. L., 266
King, N. K., 173
King, T. E., 47, 54, 55
Kingkade, M. J., 7, 25
Kinney, T. D., 295
Kipping, H., 294
Kirber, M. W., 312, 322, 326
Kirby, W. M. M., 170, 259, 261, 262
Kirchheimer, W. F., 169
Kirkwood, S., 83
Kirschner, L., 144
Kirsner, J. B., 294
Kissling, R. E., 284
Kistner, A., 107
Kitos, P. A., 78
Kitts, A. W., 290, 291, 295, 298
Kjaergaard, G., 296
Kjeldgaard, N., 249, 250, 251
Klarenbeek, A., 143
Klas, Z., 106
Klatt, O. A., 60
Klein, D. T., 209
Klein, H. P., 79
Klein, M., 168, 186, 261, 262, 375
Klein, R. M., 209
Kleinzeller, A., 450
Klemmer, H. W., 461
Klemptner, H. E., 264
Klenow, H., 82
Klepser, R. G., 364, 381
Klieneberger-Nobel, E., 23, 39, 40
Kligman, A. M., 172, 263, 374
Kline, D. L., 379
Klosa, J., 172
Kluyver, A. J., 107, 113, 114, 115
Knaysi, G., 7, 8, 10, 12, 13, 23, 24, 25, 27, 39
Kneteman, A., 435
Kniazuk, M., 169
Knight, C. A., 61, 217
Knight, S. G., 79, 249
Knisely, M. H., 274, 381
Knodt, C. B., 267
Knöll, H., 28, 38
Knowlton, K., 243, 245, 293 294, 295
Knox, W. C., 334, 335, 338
Köbe, K., 479
Koblick, D. C., 335
Koch, A., 486
Koch, A. L., 222, 244, 245, 246
Kocher, V., 48
Kodama, M., 489
Koditschek, L. K., 48, 53
Koepsell, H. J., 85
Koerber, W. L., 169
Koffler, H., 79, 114, 463
Kofman, H., 170
Kohler, A. R., 53
Kohler, R. A., 187
Kohlmiller, E. F., 120, 121
Kohn, A., 173
Kohn, K. H., 262
Kokko, U. P., 482
Kolachov, P., 85, 449, 450, 462
Kolmark, G., 221
Kolochine-Erber, B., 144,146
Komagata, K., 184
Komarova, L. I., 452, 462, 463
Komatsu, N., 172
Komp, W. H. W., 299
Kon, S. K., 258, 265, 266, 267, 458
Kondi, A., 274
Kondo, H., 283
König, H., 15
Kono, A., 462
Kooy, J. S., 438
Koppel, J. L., 72
Koprowski, H., 394, 399, 400, 401, 402, 403
Koprowska, I., 401, 402, 403, 406
Korami, T., 458
Kornberg, A., 62, 98
Korns, R. F., 298
Korthof, G., 135
Korzenovsky, M., 96
Kosaka, H., 171
Koser, S. A., 56
Koszalka, M. F., 293
Koteen, H., 296
Kozloff, L. M., 200, 202, 237, 239, 240, 241, 242, 243, 244, 245, 247
Kramer, J., 170
Krask, B. J., 57
Krauss, M. R., 208

Krebs, E. G., 76
Krebs, H. A., 80
Krebs, J., 449
Kreger, D. R., 8
Kreipe, H., 449
Krieger, C. I., 294
Krijgsman, B. J., 285
Krishnaswamy, M. A., 435
Kritschewsky, J. L., 140
Kritzler, R., 394, 396
Kroger, E., 216
Krueger, A. K., 239
Krueger, R. C., 369
Krumenacher, F. P., 263
Krupp, I. M., 280
Kruse, H., 343, 348
Kruse, R., 257, 264
Kubitschek, H. E., 223
Kucker, G., 63
Kuehn, A. O., 368
Kuh, C., 296
Kuhn, H., 136
Kuhns, D. M., 169, 171, 261
Kulp, W. L., 265, 266
Kumm, H. W., 299
Kuna, A., 382
Kunin, C., 54, 197
Kunkel, H. G , 293
Kunker, R. C., 247
Kunz, R., 452
Kurth, E. F., 461
Kurtz, H., 402
Kushner, S., 170
Kussi, P., 83
Kuwana, H., 184
Kuwata, T., 397, 398
Kwantes, C. M. S., 295

L

Labauge, R., 224
Labaw, L. W., 246, 251
Labby, D. H., 293
Labrum, E. L., 221, 222, 223
Lackey, M. D., 290, 298
Lacombe, G., 96
Lafleur, H. A., 282
Lafos, H., 452, 458
Lagunoff, D., 473
Lainson, R., 156, 158
Laird, M., 162
Laird, R. L., 159, 160, 161
Lajudie, P., 146
Lamanna, C., 32, 83, 98, 424
Lamanna, M., 25
Lambert, R. A., 488, 495
Lampen, J. O., 56, 82
Lamson, B. G., 337
Landing, B. H., 293, 344
Landman, O. E., 181-236
Landschutz, C., 401
Landsteiner, K., 357, 358
Lane, M. D., 265, 266, 267
Langbein, H., 141
Langendorff, H., 220
Langendorff, M., 220
Languillon, J., 160
Lanni, F., 199, 238, 249
Lanni, Y. T., 199, 238
Lanning, W., 382
Lansford, E. M., 54
Lapedes, D. N., 171
Lark, K. G., 194, 199
Larose, P., 9
Larsen, H., 116, 120, 121, 122, 124
Larson, A. D., 194
Larson, N. L., 266
Lascelles, J., 115, 122
Laser, H., 275
Lassiter, J. W., 267
Laster, W. R., Jr., 173
Latarjet, R., 219, 249
Lathe, G. H., 377
Latta, H., 335, 343, 344, 346
Laufer, L., 452, 459
Lauffer, M. A., 251
Laurent, A. M., 326
Lavender, A. R., 378
Laverty, J. A., 219
Lawless, D. K., 153
Lawlis, J. F , Jr., 372
Lawrence, J. J., 136, 144, 155
Lawrence, J. S., 383
Laws, J. O., 336, 346
Lawton, W. C., 452, 460, 461
Laxminarayana, H., 429
Lazarus, A. S., 314
Leach, B E., 172
Lear, S. A., 431
Leathen, W. W., 107, 109
Leaver, F. W., 88
Lebedev, A. F., 105
Leben, C., 172
LeBlanc, F. R., 172
Lechevalier, H., 172, 173
Lechner, R., 449, 452
Leder, I. G., 83
Lederberg, E. M., 24, 33, 37, 39, 40, 204, 205, 206, 207, 249
Lederberg, J., 2, 185, 188, 193, 198, 204, 205, 206, 207, 208, 249, 252, 463
Lederer, E., 124
Ledingham, J. C. G., 368
Ledinko, N., 476, 477, 481, 486
Leduc, E. H., 344, 345, 347
Lee, H. H., 222, 223
Lee, S. B., 451, 459, 462, 465
Lees, H., 92, 108, 109, 110, 111, 113, 115
Lees, T. M., 171
Legator, M., 170
Lehane, D., 295
Lehmann, J., 324
Leibowitz, S., 292, 293, 294, 296
Leidy, G., 168, 186, 209
Lein, J., 184
Lein, P. S., 184
Leloir, L. F., 84
Lembke, A., 15, 25
Lemoigne, M., 77
Lempfrid, H., 135
Lenert, T. F., 186, 261, 262
Lenhoff, H. M., 81
Lennette, E. H., 301, 430
Leonardi, G., 25
Lepine, P., 312, 313, 324, 480, 481
Lepow, I. H., 368, 425
Lepper, M. H., 259, 261, 262
Lerk, J., 436
Lerner, E. M., II, 382
Lerner, H. B., 293
Leslie, J., 1
Lesley, S. M., 240, 241, 242, 243
Lesses, M. F., 296
Lester, R. L., 94
Leupold, U., 181, 198
Levaditi, C., 154, 169, 393, 394, 395, 396, 397
Levan, A., 38
Levene, H., 214
Levenson, C. G., 493
Levey, E., 485, 491, 493
Levi-Castillo, R., 299
Levin, D. H., 62, 187
Levine, A. S , 173
Levine, H., 458
Levine, M., 188, 189, 216
Levine, P., 357, 358
Levine, S., 224
Levinthal, C , 201, 202, 241, 242, 251
Levinthal, W., 487
Levintow, L., 294
Leviton, A., 458
Levy, E., 423
Levy, J. G., 80, 293
Lewert, R. M., 159, 161
Lewin, R. A., 215
Lewis, F. S., 172, 263
Lewis, H. B , 465
Lewis, I. M., 13, 23
Lewis, K F., 79
Lewis, L. J., 481
Lewis, S. M., 121
Lewis, S. Z., 49
Libby, R. L., 345, 346, 347
Lichstein, H. C., 64, 88
Lichtenstein, M. R., 261
Lieb, M., 204, 248, 249
Lieberman, I., 62, 93, 94, 95, 98
Liebermeister, K., 206
Liebert, K. V., 62
Lieske, R., 108
Light, A. B., 312, 326
Lightbody, L. G., 438
Lijinsky, W., 458
Likely, G. D , 497
Lilienfeld, A. M., 291
Lincoln, N. S , 261
Lind, P. E., 203, 224, 318, 319
Lindan, O., 456, 457

Lindegren, C. C , 39, 190, 191, 197, 198, 211, 463
Lindegren, G., 190, 197, 198, 211
Linden, E., 63
Lindenauer, H. J., 293
Lindenfelser, L. A., 171
Lindenman, J., 219
Lindert, M. C. F., 293
Lindgren, B., 435
Lindquist, W., 451, 453
Lindstrom, E. S., 81, 121
Linge, H., 171
Lintzel, W., 454
Lipman, B. L., 293
Lipmann, F., 15, 90, 94, 95
Lips, M., 162
Lipshitz, R., 217
Litsky, W , 264, 443
Little, J. E., 172
Little, R. B., 144
Litvinova, E. V., 450, 452
Litwins, J., 296
Liu, C., 2, 10, 39
Liu, O. C., 320, 321
Lively, E. R., 40
Ljungdahl, L., 450, 452
Llewellyn-Jones, M., 411, 415, 421
Llewellyn-Smith, M., 413, 421
Lloyd, B. J., 217
Lloyd, H., 293
Lobo-Parga, G., 294
Lochhead, A. G., 63
Locke, E G., 454
Locke, F. B., 298
Lockwood, K. L , 295
Lodmell, L. A., 298
Lödöen, O., 293
Loefer, J. B., 219
Loesecke, H. W. von, 454, 458
Lofgren, N., 172
Loginova, L. G., 463
Loman, S., 143
Longcope, W. T., 338
Loosli, C. G., 312, 326, 374
Lott, H., 295
Love, R., 401, 402, 486
Lovell, R., 451
Lowenthal, J. P., 424
Lowy, R. S , 63
Lübbers, P., 140, 145
Lubbock, J., 466
Lucas, E. H., 168
Lucas, H. L., 432
Lucchesi, P. J., 49
Lucké, B., 290, 298
Lucke, W. H., 195, 218, 221
Luckey, T. D., 265
Ludány, G., 368, 382
Ludford, R. J., 496
Ludlam, G. B., 261
Ludlum, B. G., 216
Lueck, B. F., 450, 452, 462
Luecke, R. W., 267
Lumsden, W. H. R., 299
Lund, E., 260
Lundgren, H. P., 425
Lundin, H., 449, 450, 451, 452, 457
Luning, B., 172
Luria, S. E., 181, 194, 199, 201, 237, 241, 242, 246, 252, 320, 321
Luther, H. G., 267
Lutz, A., 216
Luz, E., 55
Lwoff, A., 181, 185, 195, 199, 203, 204, 248, 249, 250, 251, 273, 276, 277, 283, 284
Lyman, C. M., 48
Lynch, E. M , 376
Lynch, H. P., 261
Lynch, V., 120
Lynch, W , 261
Lynen, F., 90, 94
Lynn, J. W., 474

M

Maaløe, O., 7, 26, 32, 37, 200, 201, 241, 242, 243
Maas, W. K., 54, 94, 95, 169, 182, 197
McAllister, J., 136
Macary, J., 15
Maccacaro, G. A., 185
MacCallum, F. O., 289, 291, 292, 400, 403, 430
McCarthy, A. M., 252
McCarthy, K., 367
McCarthy, P. L., 25, 26, 27, 33
McCarty, M., 7, 294
McClelland, L., 312, 313, 324
McClelland, P. H., 375
McClure, L. E., 135
McCollum, R. W., 297
McCormick, M. H., 172
McCoy, C. M., 267
McCoy, E., 257-72; 224, 265
McCrea, J. F , 173, 377
McCutcheon, M., 380
McDonald, I. J., 440
McDougall, M. S., 273, 276
MacDowell, E , 400
McElroy, D., 49
McElroy, O. E., 424
McElroy, W. D , 92, 184, 195, 196
McFarlan, A. M., 291, 292
Macfarlane, R. G., 419, 421
McGaughey, C. A , 484
McGhee, R. B., 155, 161
McGinnis, J., 266, 267
McGinnis, R. A., 462
McGovern, J. J., 372
McGraw, J. J., Jr., 295
McGuire, J. M., 170, 173
Macheboeuf, M., 167, 337
Machlis, L., 449, 465
Machlowitz, R. A., 172
McIntyre, L. D., 107, 109
Mackal, R. P., 239, 241, 243
Mackaness, G. B., 261, 262
MacKay, D. G , 312, 326
Mackay, H., 401
McKee, D. W., 210, 211
McKee, R. W., 273, 275, 277, 283, 284
McKeen, C. L., 298
Mackenzie, H. S., 170
Mackie, T. J., 366
McKinney, G. R., 383
McKinney, R. E., 3
Maclagan, N. F., 294
McLamore, W. M., 171
McLaren, A. D., 61
MacLean, H., 262
McLean, J. R., 452, 457
Maclean, M. E., 492
McLean, R A., 10
MacLeod, C. M., 208
MacLeod, P., 443
MacLeod, R. A., 49
McMaster, P. D., 343, 348, 489
McMurtrie, E., 188
Macnamara, F. N., 299, 300
McNaught, M. L., 460
McNaughton, G. A., 477
McNee, J., 290
McNeile, H. J., 295
McOmie, J. F. W., 172
Macow, J. J., 59
MacPherson, D. A., 172, 263
McRoberts, C. C , 369
McVay, L. V., Jr., 263
MacVicar, R., 92
Macy, H., 432
Madden, S. C., 337
Maddock, H. M., 265, 266, 267
Madison, C. R., 345
Madrid, M., 294
Madsen, S., 296
Maeda, K., 171, 172
Maegraith, B. G., 273-88; 273, 274, 275, 276, 278, 279, 280, 282, 283, 284
Magaldi Jordão, F., 142
Magasanik, B., 83, 183
Magee, W. E., 93
Magill, T. P., 312, 313, 315, 316
Magne, F., 215
Magnitskaya, L. V., 109
Mahady, S. C. F., 261
Maitland, H. B., 488
Maitland, M. C., 488
Malamos, B., 156, 487
Malamut, L., 297
Mallett, V. M., 463
Mallette, M. F., 32, 79, 98
Mallmann, W. L., 443
Malmgren, H., 13
Maloney, M. A , 294
Mamelak, R., 93

Man, E. B., 294
Mande, B. A., 462
Mandel, B., 371
Mandel, W. I., 294
Mandle, R. J., 49
Mangold, F., 290, 298
Mangun, G. H., 298
Mann, J. D., 294, 295
Manresa, M., Jr., 161
Manson, E. E D., 168
Manson, L. A., 247
Manten, A., 114, 123, 183, 188, 222, 262
Mantovani, G., 142
Manwell, R. D., 154, 161, 162
Maple, F. M., 170
Marchbank, D. F., 162
Marchiafava, E., 153
Marcilla, J., 452, 462
Marcovich, H., 184, 190
Marcus, S., 368, 382
Marcuse, K., 143
Mariani, A., 7
Mariani-Marelli, O., 7
Marino, F. E., 462
Marion, D. F., 295
Markert, C. L., 195, 196, 221
Markham, R., 217
Marks, L. J., 295
Marks, V , 298
Marmell, M., 264, 265
Marmion, B. P., 430
Marnay, C., 170
Marquardt, H., 190
Marquardt, R. R., 452, 461
Marrack, J., 337
Marrack, J. R., 294
Marshak, A., 37
Marshall, A. H. E., 378
Marshall, R. O., 92
Marston, R. Q., 168
Martin, E., 167
Martin, G. J., 51, 55
Martin, N. H., 280, 294
Martin, S. P., 379, 380, 383, 384
Marvin, H. N., 276
Mason, A., 172, 263
Mason, H. L., 295
Mason, J. H., 421
Mason, R. P , 312, 313, 315, 324
Masouredis, S. P., 335, 340, 342, 346
Masters, R. E., 337
Matassarin, B. M., 294
Mathis, C., 299
Maurer, P. H., 337, 341, 342, 346, 348, 368
Maurin, J., 312, 313, 324, 494
Mauss, E. A., 355, 358
Mautner, H. G., 172
May, G., 203
Mazia, D., 25
Mean, A., 293
Meanwell, L. J., 437, 438, 439
Medalry, G. C., 290
Mefferd, R. B., 168, 219
Mehl, D. A., 168
Mehlman, B., 284
Meier, R., 380
Meiklejohn, G., 301
Meiklejohn, J., 108, 109, 111
Meister, A., 58, 93, 182, 195
Melcher, G. W., Jr., 290, 298
Melcher, L. R., 335, 340, 342, 346, 354, 355
Meleney, F. L., 262
Meleney, H. E., 280
Mellanby, E., 398
Mellanby, H., 156, 312, 326
Melnick, J. L., 293, 301, 374 431, 476, 477, 481, 486
Menke, P., 443
Menkin, V., 379
Mer, G. G., 154
Merchant, D. J., 381
Merkel, J. R., 187, 190, 196
Merrick, J. E., 443
Meschenmoser, 293
Metcalf, H. B , 170
Meter, I. van, 108
Metzgar, H. J., 431
Metzger, J. F., 171
Metzger, W. I., 264, 265
Meulemaere, J. de, 163
Mevius, W., 105, 106
Meyer, J., 108, 111
Meyer, K. F., 142, 143, 145, 487
Michael, S. R., 298
Michner, H. D., 167
Mickelson, M. N., 80
Micks, D. W., 375
Middlebrook, G., 380
Mika, L. A., 378
Milaschewskaya, W G., 421
Milatz, J. M. W., 123
Miles, D., 172
Miles, J. A. R., 291, 292
Milhaud, G., 78
Militzer, W., 11
Miller, B. F., 375
Miller, C. A., 64, 481
Miller, C. P., 263
Miller, C. S., 62
Miller, D., 246
Miller, H. K., 57
Miller, H. N., 183
Miller, I. L., 97
Miller, L. L., 337, 347
Miller, L. P., 109
Miller, P., 167
Miller, P. C , 65
Miller, S. A., 464
Miller, T. F., 144
Miller, W. N., 294
Miller, W. W., 159
Millington, R. H., 451
Mills, C. A., 382
Mills, E. H., 290, 291
Milzer, A., 262
Minck, A., 15
Minck, R., 15, 206
Mingioli, E. S., 57, 59, 92, 182
Minieri, P. P., 172
Minkenhof, J. E., 141, 143
Minsavage, E. J., 2, 36, 40, 38
Minuse, E., 311, 315
Mirick, G. S., 290, 295, 298
Mitamura, T., 487
Mitchell, C. B., 297
Mitchell, H. K., 81, 182, 183 184, 189, 190, 196
Mitchell, K. G., 266, 267
Mitchell, J. A., 443
Mitchell, L., 171
Mitchell, M. B., 81, 182, 184, 189, 190, 196
Mitchell, P., 8
Mitchell, R. B., 384
Mitchison, D. A., 186
Mitsuhashi, S., 82
Mitsui, H., 92
Mittler, S., 219
Mittwer, T., 8, 9
Miyagawa, Y., 487
Miyahara, B. T., 168, 259, 262
Mochtar, A., 143
Mocquot, G., 429
Modica, M., 294
Moewus, F., 184
Mollin, D. L., 50
Molner, J. G., 142
Monaci, V., 480, 481, 482
Moncrieff-Yeates, M., 172
Mondolfo, H., 453, 460
Monici, N., 142
Monlux, W. S., 142
Monnot, F., 191, 212
Monod, J., 57, 72, 73, 74, 193, 196, 197
Monson, W. J., 54, 267
Monuet, A., 285
Moon, V. H., 278, 279
Moore, A. C., 170
Moore, A. E., 393-410; 394, 398, 399, 401, 404, 405, 406, 473
Moore, D. J., 293, 294
Moore, E., 495
Moore, P. R., 265
Moreland, W. T., 170
Morey, G. R., 294
Morgan, H. R., 372, 381, 474, 488
Morgan, M. E., 443
Morgan, W. T., 355
Morikawa, W., 265
Morita, E., 457
Morita, S., 120
Morita, Y., 280
Morowitz, H. J., 219
Morris, D., 296, 297
Morris, H. A., 440, 442
Morris, M , 462
Morrison, D. B., 279

Morse, I., 252
Mortenson, L. E., 93
Mortimer, D. C., 167
Mortimer, R. K., 222
Morton, H. E., 3, 5, 134
Morton, H. S., 264
Morton, J., 170
Moseley, O., 48
Mosley, V M., 251
Moss, J., 51, 55
Moualim, R. J., 170
Moulder, J. W., 56, 168, 283, 284, 384
Moulder, P. V., 298
Moulton, B., 259, 261
Mount, F. W , 261
Mountain, D. C., 263
Moyer, V. A., 355
Moyle, J., 8
Moyle, J. M., 8
Muckenfuss, R S , 488, 489, 490
Mudd, S., 1-22; 1, 2, 3, 7, 10, 11, 13, 15, 24, 25, 27, 32, 33, 200, 237
Mudrow, L., 153, 159, 160, 161
Mueller, J. H., 58
Mukai, F., 199, 222
Mulder, H., 429
Mulder, J , 312, 313, 314, 324
Mull, R. P., 450, 452, 454, 461
Mullady, H. E., 171
Mullaney, J., 220
Muller, A. E., 452, 454
Müller, H., 105
Müller, P., 450, 451
Müller, R., 133
Müller, T., 293
Muller, W. R., 452, 460
Mullett, P. A., 430
Mulligan, H. W., 155
Mulliken, B., 394, 396, 401
Mundkur, B. D., 190, 211
Muniz, J., 162, 163
Munns, D. J., 452, 454, 464
Murayama, M., 384
Muri, J., 296
Murphy, F. D., 275
Murphy, H. W., 494
Murphy, J. S., 202, 494
Murphy, M. K., 251
Murphy, T. P., Jr., 364
Murphy, W. P., Jr., 298
Murray, E. G. D., 264
Murray, E. S., 374
Murray, R., 296, 297, 298
Murray, R. G. E., 7, 24, 25, 27, 117, 200, 246, 249
Murzaev, P. M., 109
Muscatine, N. A., 194, 199
Mutsaars, W., 201
Mylroie, A , 221, 222
Myrback, K., 450, 452
Myrvik, Q. N., 370

N

Nadson, G., 109
Nagel, L., 13
Nagler, F. P., 312, 313, 324
Nair, C. P., 156
Najjar, V. A., 92
Nakamura, H., 115, 184
Nakaya, R., 6
Nanney, D. L., 192, 215
Napier, L. E., 284
Nashif, S. A., 433, 441
Nason, A., 49, 92, 184
Nathansohn, A., 112
Nauck, E. G., 484, 487
Nawrocki, J., 355
Naylor, J. M., 461
Neckel, I., 15
Needham, G. M., 257, 258, 259, 260, 261, 264, 265, 372
Neefe, J. R., 294, 297
Neilands, J. B., 48
Neish, A C., 83, 464
Neithammer, A., 450, 451, 452
Nélis, P., 140
Nelson, C. I , 412
Nelson, E. V., 183
Nelson, F. E., 240, 433, 438, 439, 441, 442
Nelson, J. B., 290, 298
Nelson, R. E., 240
Nelson, R. S., 294, 295
Nelson, T. S., 267
Nelson, W. O., 54, 441, 442
Nerenberg, S. T., 365
Nermut, M., 7
Nesbitt, S., 293
Neu, H., 452, 462
Neuberg, C., 169, 457
Neufeld, E. F , 74
Neva, F. A., 483, 484
Newcombe, H. B., 222, 223
Newcomer, E. H., 25
Newland, L. G. M., 434
Newman, B., 294
Newman, J. P., 143
Newman, N. H., 296
Newmeyer, D., 197, 210, 212
Newton, B. A., 171
Newton, G. G. F., 167, 171, 172
Newton, J. W., 92, 121
Ney, F. G., 365
Nice, S. J., 195, 204
Nichol, C. A., 52, 53, 54
Nicholas, D. J. D., 49, 92
Nichols, D. R., 258, 259, 260, 261
Nicholson, L. W., 85
Nickerson, W. J., 190
Nicolajev, B. P., 158
Nicolau, S., 394, 395, 396
Nicolle, P., 193, 200, 239
Nicosia, J., 299
Niel, C. B. van, 105-32; 116, 118, 119, 120, 122, 123
Nielsen, A. J., 440
Nielsen, N., 450, 452, 454
Niemegeers, L., 137, 138
Nijenhuis, L. E., 123
Nikoul, E , 25
Nilsson, R., 450, 452
Nimmo-Smith, R. H., 53
Nisman, B., 96
Nitta, K., 172
Niven, C. F., 53
Noble, K., 186
Nobrega, P., 142
Nocke, E., 296
Noda, H., 237
Nooyer, I. De, 314
Nord, F. F., 450, 452, 454, 461
Norkrans, B., 193
Norman, A., 194, 220
Noronha-Peres, J., 358
Norris, L. C., 265
Norris, R. F., 297
Northcote, D. H., 8
Northrop, J. H., 202, 205
Norton, T., 394, 399, 400
Norton, T. W., 486
Novel, E., 195, 221
Novelli, A., 3
Novelli, G. D., 94, 95
Noyes, H. E., 170, 261
Noyes, W. F., 489
Numerof, P., 167
Nungester, W. J., 363-92; 364, 379, 381, 382
Nutini, L. G., 376
Nybom, N., 215, 218, 219, 220
Nye, R. N., 488, 490
Nygaard, A. P., 437

O

Oakley, C. L., 411-28; 411, 413, 414, 415, 419, 421, 423
Obayashi, A., 290, 298
Oberlé, G., 160
O'Brien, R. A., 421
O'Connor, S., 398
Odin, M., 296
Oginsky, E. L., 50, 96, 167, 168, 170
Ogur, M., 190
Oikawa, K., 171
Okajima, M., 56, 169
Okami, Y., 171, 172
Okanishi, J., 487
Okell, C. C., 422
Okono, C., 421
Olcott, H. S., 167
Oldewurtel, H. A., 184
O'Leary, B., 170
Olejnik, E., 144
Oleson, J. J., 49, 170, 265
Oliphant, J. W., 297, 298
Olitsky, P. K., 366, 369
Olitzki, A. L., 146, 169, 188
Olitzki, L., 144, 145

Olive, L. S., 210
Oliver, W. H., 437
Oliver-Ganzales, J., 353-62; 354, 355, 356, 357, 358, 359, 360
Olson, B. H., 172, 464
Olson, J. C., Jr., 432, 440
O'Malley, E., 86
Omata, R. R , 63
Oncley, J. L., 366
Oppenheimer, J. R., 125
Opperman, A., 292
Orbán, T., 382
Ordman, C. W., 296
Orlans, E. S., 369
Ormerod, J. G., 119
Ornstein, L., 25, 26
Ortiz, L., 266, 267
Osman, H. G., 122, 123
Östergren, G., 25, 33
Ostertag, H., 142
Ostrowskaya, O. A., 421
Ottinger, B., 337
Otzen, D., 109
Ouchterlony, Ö., 423
Oudin, J., 423
Overcast, W. W., 437, 438
Overman, J. R., 364
Overman, R. R., 275, 279, 284, 285
Owades, P., 57
Oyaas, J. E., 458
Oyama, J., 337
Oyama, V. I., 64

P

Pack, G., 405
Packer, L., 64
Pagano, J. F., 169
Paine, R. F., 168
Paine, R. S., 296
Paine, T. F., Jr., 372
Paley, S. S., 296
Palleroni, N. J., 197
Palm, L., 246
Palm r, W. L., 294
Pan, S. C., 85, 167, 449, 450, 462
P'an, S. Y., 171
Pansy, F. E., 169, 171
Paoletti, A., 371
Papazian, H. P., 209
Pappenheimer, A. M., 185, 205
Pappenheimer, A. M., Jr., 73, 422, 424, 425
Pappenport, R. B., 263
Paraense, W. L., 161
Pardee, A. B., 15, 117, 118, 125, 201, 247
Parent, M., 163
Parke, T. V., 86
Parker, A., 463
Parker, C. D., 110, 112
Parker, E., 364
Parker, F., Jr., 488, 490
Parker, R. B., 185, 438, 439, 440
Parmelee, C. E., 438, 439
Parodi, A., 403
Parrott, R. H., 372
Parrott, T. H., 476, 484
Parry, W. R., 248
Partridge, C. W., 183, 198
Pask, V. M., 146
Paul, J. R., 292, 296, 301
Pavcek, P. L., 452, 460, 461
Paxton, J. A., 440
Payne, A. M. M., 282, 312, 325
Payne, J. I., 2, 25
Pearce, L., 396, 399, 400
Pearse, A. G. E., 383
Peck, H. D., Jr., 79, 80
Pecora, L., 267
Peebles, T. C., 492, 493
Peeters, E., 163
Pelczar, M. J., 63
Pendergrass, G. P., 267
Pennell, R. B., 297, 298
Pennington, D., 122
Pennington, F. C., 171
Pepinsky, J. B., 452, 460
Pepper, B. O., 292
Pepper, W. F., 265, 459
Peppler, H. J., 464
Pereira, J. M., 160
Perez-Reyes, R., 163
Perkins, A. B., 56
Perkins, D. D., 210
Perlman, D., 171, 464
Perret, C. J., 167
Perrins, T. D., 251
Perry, M. F. C., 218
Perry, W. D., 382
Perry, W. L. M., 156
Pessin, V., 442
Peters, I. I., 442
Peters, J. P., 294
Peters, V. J., 60, 61
Peterson, G. E., 265, 266
Peterson, R. E., 294
Peterson, W. H., 167, 452, 454, 455, 458, 460, 461, 462, 464
Petersson, A., 368
Petisi, J. P., 170
Petrakis, N., 405
Petronelli, A., 25
Petrow, V., 170
Pette, J. W., 438
Pfeifer, V. F., 459
Pfennig, N., 171
Phares, E. F., 87, 88
Philipp, E., 141
Phillips, A. P., 170
Phillips, G. E., 454, 457, 458
Phillips, R. L., 463
Picard, J., 145
Pick, F., 154
Pickett, M. J., 186
Pidacks, C., 48
Piekarski, G., 23, 24, 134
Pierce, C. H., 380
Pijper, A., 6
Pikovski, M., 497
Pikula, D., 261
Pilgrim, F. J., 170
Pillemer, L., 366, 368, 424, 425
Pinchot, G. B., 81
Pinfield, S., 426
Pinsky, M. J., 121, 463
Pintner, I. J., 49
Pinto, M. R., 160
Pirosky, I. R., 354, 355
Piskur, M. J., 457
Pital, A., 168
Pittenger, R. C., 170, 224
Pittenger, T. H., 198, 212, 218
Pittillo, R. F., 54, 65, 168
Pittman, D. D., 190, 191
Plankenhorn, B., 6
Plotz, H., 485
Plough, H. H., 183
Plough, H. P., 252
Pochon, J., 107
Pohler, G. M., 464
Pohlmann, R., 143
Polgár, A., 122, 123
Poliard, M., 294, 404, 494, 495
Pollock, M. R., 72, 73, 74, 168, 196, 291, 292
Pomper, S., 54, 57, 182, 210, 211, 463
Pond, W. L., 364
Ponte, E. del, 299
Pontecorvo, G., 182, 213, 214, 216, 221, 224, 463
Poor, W., 495
Pope, C. G., 415, 422, 423, 424, 425, 426
Popp, L., 143
Popper, H., 294
Porath, J., 171
Porges, N., 452, 460
Porter, C. J., 72
Porter, J. E., 296
Porter, J. R., 458
Porter, J. W. G., 258, 265, 267, 458
Posner, I., 93
Post, J., 293
Posternak, T., 83
Postgate, J. R., 107, 109, 111, 113
Poth, E. J., 257, 258, 264
Potter, N. N., 240, 438
Potter, V. R., 171
Pottinger, M. A., 220
Pounden, W. D , 267
Powell, H. M., 170, 173
Powelson, D., 114
Power, M. H., 295
Powers, E L., 219
Pratt, D. D , 109
Pratt, R., 167, 168, 170, 172
Preer, J. R., 189

Prescott, G. E., 170
Prescott, J. M., 60, 61
Preston, W. S , 384
Preuner, R., 10
Preuss, L. M., 458
Price, A., 402
Price, S A., 421
Price, W H., 237, 364
Prichard, M. M. L., 278, 284
Pridham, T G., 171, 459
Pringsheim, E. G., 23, 107, 112, 117, 122
Prisk, J., 110, 112
Prittwitz und Gaffon, J. v., 10, 206
Prophet, A. S., 421
Propp, S., 263, 372
Prout, T., 214
Prouty, C. C., 430, 438, 439
Provasoli, L., 49, 50
Pruess, L. M., 48
Pruitt, F. W , 294, 295
Puck, T. T., 184, 200, 224, 239, 240
Pugh, L. H., 173
Puglisi, T. A., 184
Pulaski, E J., 170, 261
Pumarola Busquets, A., 136
Purcell, E. M., 168, 170, 188, 259, 262
Purdie, I. A., 260
Purko, M., 54, 442
Putnam, F. W., 237, 240, 243, 244, 245, 246

Q

Quastel, J. H., 61, 93, 105, 109, 110, 111, 113
Quesnel, J. J., 163
Quilligan, J. J., Jr., 311, 312, 315, 326
Quinn, L. Y., 265, 266, 267
Quintini, J., 25
Quiring, E., 169
Quisenberry, J. H., 267
Quispel, A , 109

R

Rabe, E. F., 370
Rabinowitz, J. C., 55
Rabotnova, I. L., 109
Rackemann, F. M., 338
Racker, E., 83, 371
Radcliffe, H., 290, 298
Radike, A. W., 285
Raettig, H., 202
Raffaele, G , 153, 154, 159
Rafols, W. de, 462
Rafsky, H. A., 294
Rafter, G W., 76
Ragan, C., 290, 298
Raghavendra Rao, M. R., 64
Raistrick, H., 451
Ramon, G., 422
Randall, C. C , 485
Randall, R., 140, 142
Randall, W. A., 170
Ranson, S. E., 430
Ranson, S. L., 118, 119
Rao, M. R. R., see Raghavendra Rao, M. R.
Rao, V. G., 283
Raper, J. R., 215, 222
Raper, K. B., 172, 214, 463
Rapkine, S., 250
Rapoport, S., 81
Rappaport, C., 194, 199
Rappaport, E. M., 294
Rappaport, I., 185
Rapuzzi, A. L., 171
Raskin, H. A., 142
Rasmussen, A. F., Jr., 312, 326, 364
Ratner, S., 337
Ratnoff, O. D., 295, 368
Rauch, J., 450, 451, 452
Raut, C., 183, 184
Ravaioli, L., 144
Ravin, A. W., 194
Ravin, H. A., 294
Ray, A. P., 156
Ray, H N., 154, 156
Rayman, M. M., 442
Raynaud, M., 412
Razumov, A. S., 107
Rdzok, E. G., 171
Rdzok, E. J., 171
Reagan, R. L., 301
Rebstock, M. C., 170
Rebuck, J. W., 383
Redman, W., 209
Reed, G., 459
Reed, L. J., 86
Reed, R., 321
Reed, R. W., 142
Reedy, R. J., 170
Reese, E T., 452, 463
Regna, P. P., 170, 171
Reichenow, E., 160, 161
Reid, B. L., 267
Reid, E. A. S., 142
Reif, A. E., 171
Reifenstein, R. W., 294
Reiman, J., 382
Reinartz, F , 452, 458
Reinbold, G. W., 443
Reinertson, J. W., 283
Reinhardt, W. O., 457
Reinhold, J. G., 297
Reinie, L., 312, 313, 324, 393, 397
Reio, L., 59, 119, 121
Reisner, A. R., 212
Reissig, J. L., 95
Reller, H. H., 346, 347
Reman, G. H., 122
Renneberg, K., 172
Renella, E., 145, 146
Resemann, G., 292
Reymond, D., 83
Reynaud, R , 285
Reyniers, J. A., 365
Reynolds, E., 442
Reynolds, W. E., 294
Reynolds, W. M., 267
Rhodes, A. J., 322, 324, 477, 488
Rhodes, R. A., 267
Rhodes, R. E., 171
Rhumball, J. M., 295
Ribas, J. C., 142
Rice, C. E., 367, 368
Rice, E. C., 372
Richards, H. J. A., 292
Richardson, G. M., 48
Ricketts, W. E., 294
Riddell, M. I., 264
Riel, J. van, 142, 143, 144, 146
Rifkin, H., 295
Rigdon, R. H., 274, 276, 278
Rigler, N. E , 172
Riley, D. P., 217
Rimpau, W., 133, 137, 140, 143, 144, 145, 146
Rinjard, P., 394
Riordan, J. T., 476, 477
Rippel, A., 450
Rissel, E., 295, 296
Rittenberg, D., 337
Rittenberg, S. C., 111
Ritz, E., 195, 203, 204
Rivera, J. A., 295
Rivers, T., 396, 399, 400
Rivers, T. M., 488, 489, 490
Rivoire, Z. C., 261
Rizet, G., 191, 192, 198, 212
Robbins, F. C., 473, 476, 477, 480, 482
Robbins, W. J., 50, 188
Roberts, C., 198, 211
Roberts, E., 58, 93
Roberts, J. C., 172
Roberts, L. P., 459
Roberts, R B., 80, 95
Roberts, R. H., 296
Roberts, S. J., 144
Roberts, W. S., 172
Robertson, A., 172
Robertson, A. H., 442
Robertson, J. S., 343
Robertson, O. H., 374
Robinow, C. F., 7, 8, 23, 24, 26, 27, 32, 36, 38, 39, 41, 488
Robinson, E. S., 422, 425
Robinson, F. O , 159
Robinson, H. J., 169
Robinson, J., 93
Robinson, J. W., 144, 295
Robinson, R. F., 449, 454
Roche, J., 96
Rochford, E. J., 49
Rodaniche, E. C. de, 299
Rodgers, R. D., 224
Rodhain, J., 155, 162, 13
Rodwell, A. W., 51, 96
Roetner, F. R., 167
Roelofsen, P. A., 121, 126

Rogers, D. E., 385
Rogers, N. G., 312, 313, 324
Rogers, W. I., 172
Rogick, F. A., 440
Rogosa, M., 443
Rolly, H., 40
Roman, H., 210
Romansky, M. J., 169
Romoser, G. L., 266
Rooyen, C. E. van, 240, 241, 242, 243, 312, 313, 322, 324
Roper, J. A., 213, 463
Rosanoff, E. I., 65, 169, 187
Rose, C. S., 456
Rose, D., 440
Rose, W. C., 452, 465
Rosenberg, B. L., 143
Rosenberg, M., 296
Rosenberg, M. M., 265
Rosenblum, H., 373
Rosenow, G., 380
Rosenthal, N., 298
Ross, E. B., 267
Ross, G. I. M., 50
Ross, H. E., 411, 413, 414, 415, 421
Ross, S., 372
Rossi, P., 144
Rossiter, R. J., 383
Rossnoff, I. S., 267
Rostorfer, H. H., 274, 278
Roth, L., 247
Roth, R., 202
Rothbard, S., 367
Rothenbach, E., 463
Rotman, B., 190, 196
Rothman, S., 374
Rotman, R , 318
Rotwitt Schmidt, M., 145
Rountree, P. M., 7, 239
Rous, P., 489
Routh, J. I., 294
Routien, J. B., 171
Rovelstad, R. A., 293, 294
Rowe, W. P., 476, 484
Rowlands, A., 429, 430
Rowley, D., 58, 183, 222
Rozansky, R., 373
Rubin, B. A., 218
Rudert, F. J., 464
Rudman, D., 58, 93, 182, 195
Ruggieri, P., 295
Rundles, R. W., 383
Runyan, J., 297
Ruppert, A., 451
Ruska, H., 15, 134
Russell, P. F., 153
Ruth, H. J., 168
Ruthven, C. R. J., 377
Rutter, W. J., 84
Ruyle, E. H., 433
Ruys, A., 142, 145
Ryan, F. J., 54, 197, 214, 221, 222
Ryder, F. W., 485
Ryser, F. C., 438

S

Sabachewsky, L., 401
Sabin, A. B., 300, 301, 302, 486
Sabin, F. R., 342
Sacks, L. E., 172
Saddington, R. S., 495
Sadum, E. H., 280, 282
Saeman, J. F., 454
Sager, R., 215
Sagik, B., 200, 224, 239
Sah, P. P. T., 169
Sahyun, M., 455
Sakamoto, Y., 91
Salamon, I. I., 59, 92
Salk, J. E., 311, 312, 326, 481, 482
Salmon, P., 404, 405
Salmon, W D , 456
Salton, M. R. J., 7
Saluste, E., 59, 119, 121
Salvesen, H. A., 293
Sampaio, A. A. C., 313
Samuels, P. J., 94
Samuels, S. L , 414
Sandegren, E., 450, 452, 454
Sanders, E. P., 283
Sanders, M., 295, 473, 487
Sanders, T H., 63
Sands, S. M., 210
Sanford, K. K., 497
Santos, M., 142
Sapin-Jaloustre, H., 142
Sarachek, A., 190, 195, 218, 221
Sarles, W. B., 249, 265, 267
Saroff, A. H., 162
Sartory, A., 108, 111
Sartory, R., 108
Sartwell, P. E., 291
Sato, R., 183
Sauberlich, H. E., 53
Sauerwein, E., 296
Saurino, V., 492
Sauthoff, R., 401
Sautter, V., 312, 313, 324
Savage, N., 6
Savard, K., 458
Savino, E., 145, 146
Saz, A. K., 76, 170, 221
Sborov, V. M., 291, 292, 294, 295, 296
Scaletti, J. V., 25, 265, 266
Scanga, F., 7, 25
Scardovi, V., 117, 121
Schabel, F. M., Jr., 173
Schachman, H. K., 15, 117, 118, 125
Schaechter, M., 26
Schaefer, W. B., 169
Schaeffer, P., 169, 184, 195
Schaiberger, G. E., 187
Schapira, R., 476, 477, 491, 494
Schär, B., 380
Schatz, A., 107, 114, 115
Schaub, R. E., 172
Schaudinn, F., 40
Schayer, R. W., 344
Scheele, L., 137
Scheiffley, C. S., 296
Scheinberg, I. H., 295
Schell, F. S., 485
Schenck, G. O., 125
Schenck, J. R., 171
Schendel, H. E., 458
Scherer, W., 403
Scherer, W. F., 404, 476, 479, 480, 481, 482, 486, 491
Schermer, J., 143
Schieler, L., 135
Schiller, A. A., 344
Schingarew, A., 154
Schlegel, H. G., 114, 115
Schlenk, F., 96
Schlesinger, R W., 301, 302
Schlipköter, H. W., 135, 147
Schlossberger, H., 133-52; 133, 134, 137, 141, 144
Schmid, G., 144
Schmidt, G., 14, 15
Schmidt, L H., 156
Schmidt, M., 449
Schmidt, M. R., see Rotwitt Schmidt, M.
Schnakenberg, G. H. F., 86
Schnall, E. S., 263
Schnayerson, A. F., 414
Schneider, B. H., 266
Schneider, H. A., 364
Schneider, M. D., 138
Schneider, P., 380
Schneiderman, A., 135
Schnellen, C. G. T. P., 107, 115
Schnitzer, R. J., 262
Schoch, W., 429
Schoen, R., 154, 393, 394, 395, 396, 397
Schoenars, F., 142
Schoenbach, E. B., 294
Schoenberger, J., 312, 326
Schoenhard, D. E., 171
Schoenheimer, R., 377
Schoenherr, K. E., 140, 142, 143, 147
Scholefield, P. G., 105, 110, 111, 113
Schonfeld, W., 354, 357
Schoop, G., 144
Schörmuller, J., 451, 452
Schorr, S. E., 168, 186, 261, 262
Schrack, W. D., Jr., 291
Schramm, G., 217
Schrek, R., 170
Schüffner, W., 137, 142, 143
Schule, L., 490
Schuler, M. N., 80
Schulman, J. H., 171
Schussing, B., 42
Schuster, G., 15
Schwartz, D. R., 452, 459

Schwartz, L., 39
Schwartz, M., 57
Schwarz, K., 457
Schwerdt, R. F., 77, 78
Schwertz, J., 154, 161
Scott, D. B. M., 79
Scott, K. W., 267
Scott, L. C., 280
Scott, T. F. McN., 491
Scrimshaw, P. W., 162
Scudamore, H. H., 294
Sculthorpe, H. H., 274, 278, 283
Seaman, G. R., 86
Second, L., 412
Seegmiller, J. E., 90
Seeley, R. D., 455
Seifter, S., 366, 368
Seligman, A. M., 294, 334, 337
Seligmann, E., 202, 263
Seligson, D., 295
Sellers, E. S., 462, 465
Sellers, K. C., 141
Sen, K. C., 429
Seneca, H., 171
Senekji, H. A., 354, 355
Senez, J., 107, 109
Sen Gupta, P. C., 285
Sensi, P., 170
Seraidarian, K., 63
Sergent, E(dmond), 153
Sermonti, G., 213, 463
Seshachar, B. R., 215
Setlow, R. B., 219
Seto, J., 262
Sevag, M. G., 65, 90, 169, 171, 187
Sfat, M R., 464
Shaefer, A. E., 456
Shaffer, F. W., 312, 326
Shaffer, J. M., 295
Shapiro, A. L., 374
Shapiro, S. K., 57, 182, 183
Sharp, E. S., 85
Sharpless, G., 395, 399
Shaw, E., 378
Shee, J. C., 364
Sheffner, A. L., 49, 294
Sher, I. H., 79
Sherman, J. H., 171
Sherrard, E. C., 452
Sherwood, P. M., 296
Shevky, M. C., 186, 262
Shibuya, M., 370
Shiguera, H., 59
Shimiga, S., 487
Shimkin, M. B., 342, 346, 405
Shipe, W F., Jr., 442
Shiroishi, T., 162
Shive, W., 53, 54, 56, 59, 62
Shneyerson, S., 144
Shockman, G. D., 267
Shope, R. E., 173, 324
Shorb, M. A., 63
Shorb, M. S., 266
Shore, C., 294
Short, E. I., 171
Shortt, H. E., 156, 157, 158, 159
Shotwell, O. L., 171
Shrivastava, R. K., 451
Shu, P., 464
Shumacker, H. B., Jr., 375
Shunk, C. H., 173
Shurter, R. A., 464, 465
Shute, P. G., 156, 157
Sicher, S., 59, 170
Siddiqi, M. S. H., 243, 244
Sideri, C. N., 167
Sieburth, J. M., 266
Siegel, A., 16
Siegel, A. L., 185
Siegel, J. M., 118, 119, 121
Siegel, M., 172, 349
Siegel, R. W., 188, 189
Siemienski, J., 170
Sigel, M. M., 312, 326
Sigurdsson, B , 142
Sigurdsson, P., 142
Sijderius, R., 108
Silberstein, H. E., 337
Silver, W. S., 184, 195, 196
Silverthorne, N., 477
Siminovitch, A , 185, 203
Siminovitch, L., 72, 205, 247, 249, 250, 251
Simmonds, S., 57, 59, 60, 453
Simmons, G. C., 144
Sineath, H. H., 465
Singer, S., 62, 187
Singer, S. J., 16
Singer, T. P., 97
Singh, J., 156
Singh, K., 452, 462, 464
Singleton, J. R., 212
Sinnamon, C. N., 146
Sirny, R. J., 54
Sisler, F. D., 107, 109
Sistrom, W. R., 91
Siu, R. G. H., 452, 463
Sjöstrand, F. S., 7, 26, 32, 37
Skeggs, H. R., 62
Skinner, C E., 452, 454
Skinner, C. S., 451, 452, 454
Skinner, J. T., 452
Skipper, H. E., 173
Skolnick, M., 364
Skrodzki, E., 142
Slade, H. D., 96
Slamp, W. C., 96
Slater, E. C., 85
Slatter, W. L., 433
Slie, R. B., 76, 170
Slinger, S. J., 265, 266, 267, 459
Sloan, J. Q., 298
Slonimski, P. P., 184, 190
Smadel, J. E., 312, 326
Smetana, H., 343
Smiljanic, A. M., 374
Smith, A. G., 24, 25
Smith, B. F., 296
Smith, C. E., 458
Smith, C. K., 267
Smith, C W., 294
Smith, D. J. W., 146
Smith, E. L., 458
Smith, F., 168
Smith, G. N., 167, 168, 170
Smith, H., 382
Smith, H. H., 298
Smith, J., 429
Smith, J. D., 247
Smith, M. R., 380, 382
Smith, N., 261, 262
Smith, P. H., 50
Smith, R A , 81
Smith, R. L., 96
Smith, S. C., 364
Smith, V. N., 185, 438, 440
Smith, W., 312
Smith, W. H., 203, 295, 296
Smith, W. M., 478, 480
Smith, W. W., 168
Smithburn, K. C., 298, 299
Smithies, W. R., 452, 453
Smolens, J., 188
Smyrniotis, P. Z., 82
Smythe, C. V., 459
Smythe, M. P., 116
Smythe, V. R., 438
Snavely, J. G., 298
Snell, A. M., 292
Snell, E. E., 48, 49, 54, 55, 56, 60, 61
Snell, J. F., 461, 464
Snipes, A., 399
Snodgrass, H. M., 293
Snoke, J. E., 94
Snyder, F. H., 374
Snyder, J. C., 374
Soares, R., 162, 163
Soars, M. H., 50, 53
Sobin, B. A., 171
Soeda, M., 172
Sognefest, P., 433
Sokatch, J. T., 78
Solberg, P., 429
Solomon, C., 295
Solomons, I. A., 171, 172
Solotorovsky, M., 169
Soltero, F. V., 167
Sommermeyer, K., 220
Sommerville, T., 155
Sonkin, L. S., 365
Sonn, C., 372
Sonnenfeld, V., 92
Sonoda, Y., 172
Soo Hoo, C. M., 342
Soper, F. L., 299
Soper, Q. F., 86
Sörensen, H., 111
Sorouri, P., 11
Soska, J. W., 259, 261, 262
Sourkes, T. L., 57
Southam, C., 400, 401, 404, 405, 406
Specht, H., 463
Speck, M. L., 432
Spector, B. K., 281

Spellberg, M. A., 294
Sperber, E., 451
Spicer, D. S., 62
Spicer, S., 372
Spiegelman, S., 181-236; 14, 72, 73, 190, 196, 199, 209
Spier, V. C., 267
Spies, H. W., 259, 261
Spies, T. D., 378
Spilman, W., 208
Spink, W. W., 264, 372
Spivey, M. R., 266, 267
Spizizen, J., 298
Sprent, F. J., 354, 357
Sprince, H., 63
Springer, R., 452, 460
Sprinson, D. B., 59
Sprinz, H., 294, 295
Spruit, C. J. P., 109
Sprunt, D. H., 263
Spurr, E. D., 47
Squadrini, F., 141
Srb, A. M., 184, 214
Sreenivasaya, M., 462
Sreepathi Roa, S. K., 211
Ssilanowa, I. W., 421
Stabler, R. M., 280
Stacey, M., 9, 371
Stadhouders, J. J., 429
Stadler, J., 183
Stadtman, E. R., 90, 94
Stadtman, T. C., 105
Stafseth, H. J., 168, 171
Stahl, J., 293
Stahmann, M. A., 61
Stakman, E. C., 463
Stander, H., 169
Stanier, R. Y., 15, 77, 91, 97, 110, 117, 118, 125, 196, 216
Stapleton, G. E., 219, 220
Stare, F J., 465
Stark, P. S., 189
Stark, W. H., 464
Starkey, L. R., 450
Starkey, R. L., 109
Starr, M. P., 6
Starr, T. J., 81, 183
Stary, Z., 124, 458
Staszko, P., 450, 452
Staub, H., 169
Stauffer, J. F., 167
Stearman, R. L., 54
Stebbins, M. E , 50, 118
Stebbins, M. R., 486
Steele, H. H., 297
Steele-Bodger, A., 264
Steenboch, H., 452, 455, 454, 458
Steenken, W., Jr., 216, 261
Steers, E., 186, 187, 196
Steffano, H. S. di, 25
Steffee, C. H., 382
Steigman, A. J., 290, 482
Steigmann, F., 294, 295, 296
Steigner, K. F., 143
Stein, J. F., 267
Stein, W., 194, 220, 221
Steinberg, B., 11
Steinberg, D., 296, 297
Steinhardt, D., 495
Steinhardt, E., 488
Steinman, H. G., 64
Stempel, B , 257, 260
Stempen, H., 28
Stent, G. S., 200, 201, 203, 218, 223, 240, 242
Stephens, C. R., 170
Stephenson, E. L., 267
Stephenson, O., 170
Sterling, K , 294, 335, 337, 341
Stern, C., 213, 214
Stern, H., 17
Stern, J. R., 266
Stern, K G., 294
Stern, R. M., 458
Sterne, M., 3
Stessel, G. J., 172
Stevens, C. M., 221, 222
Stevens, K. M., 167, 348
Stevens, M. F., 414, 421, 422, 423, 424, 425, 426
Stevenson, I. L., 110
Stevenson, J. W., 113
Stevenson, W. J., 144
Stewart, E. D., 452, 459
Stewart, G. T., 283
Stewart, R. C., 90
Stich, H., 192, 215
Still, H., 296
Still, J. L.,115
Stille, B., 23
Stillians, A. W., 264
Stirn, F. E., 170
Stjernholm, R., 59, 119, 121
Stock, C. C., 189, 401
Stocker, B. A. D., 2, 198, 208, 252
Stoddard, M. B., 476, 477, 482, 491, 495
Stoenner, H. G., 140
Stoerk, H. C., 364
Stokes, A. R , 217
Stokes, F. N., 77
Stokes, J., Jr., 290, 291, 295, 296, 297, 298
Stokes, J. C., 312, 326
Stokes, J. L., 47, 64, 107, 463
Stokstad, E. L. R., 49, 258, 265, 267
Stoler, M., 404, 481
Stone, D., 48
Stone, J. D., 316
Stone, R. L., 170
Stone, R. W., 167
Stone, W. S., 168, 219, 222
Stones, 300
Storm, J., 50
Story, C. D., 265, 266
Stout, H. A., 114
Stovall, J. C., 295
Strassman, M., 96, 451
Stratman-Thomas, W. K., 274
Strauss, B. S., 86, 197
Strehler, B. L., 92
Streightoff, F., 170
Streitfeld, F. H., 293, 294
Strength, D. R., 456
Striegler, E., 478
Strode, G. K., 298
Ström, J., 294
Strong, F. M., 171
Strong, P. S., 367
Strumia, M. M., 295
Stuart-Harris, C. H., 312, 324, 322
Stuczynski, L. A., 144, 145
Stulberg, C. S., 476, 477, 491, 494
Stumbo, C. R., 172
Sturdy, J. H., 312, 313, 324
Sturgeon, B., 170
Subramanian, M. K., 211
Sugg, J. Y., 312, 316, 317
Sugihara, T. F., 462
Sugiyama, T., 290, 298
Sulkin, S. E., 364
Suminoe, K., 452, 462
Sumner, R. J., 455
Sunakawa, S., 6
Sung, S. S., 337
Supinska-Jakubowska, J., 435
Sure, B., 452, 455
Suriano, P. C., 311, 326
Suskind, R. R., 374
Sussman, A. S., 195, 196
Sussman, M., 184, 187
Sussman, R. R., 184
Suter, E., 386
Sutherland, A. K., 144
Sutherland, D. A., 294, 295
Sutliff, W. D., 298
Sutton, W. B., 98
Suzuki, K., 120
Suzuki, N., 462
Swanzon, A. L., 170
Swartling, P., 434, 435
Swartzwelder, J. C., 280, 281
Swendseid, M. E., 170
Swern, M., 443
Swick, L., 372
Swift, W. E., Jr., 293, 294
Swim, H. E., 88
Swift, W. E., Jr., 293, 294
Sykes, C., 298
Sylvester, J. C., 65, 170, 171
Symons, J. M., 439
Symonds, N., 201, 241
Synge, R. L. M., 115, 377
Syverton, J. T., 404, 476, 479, 480, 481, 482
Szafarz, D., 192, 215
Szaflerski, J., 355
Szumski, S. A., 441
Szybalski, W., 2, 36, 40, 169, 186, 187, 188, 196, 262
Szendy, S. R., 171

T

Tabachnick, M., 121
Tagle, V. I., 355
Tagliani, N. J., 171
Takahashi, B., 172
Takamiya, A., 88
Takashima, S., 120
Takata, O., 462
Takata, R. J., 451, 452, 454
Taketomo, Y., 91
Takeuchi, T., 172
Taliaferro, L. G., 156, 348
Taliaferro, W. H., 156, 279, 284, 348, 356, 374, 375
Talmage, D. W., 336, 337, 339, 340, 341, 346, 348, 368
Tamamura, Y., 290, 298
Tamelen, E. E. van, 171
Tamita, K., 83
Tamm, I., 173, 314, 317
Taniguchi, S., 92
Tannenbaum, S. W., 79
Tannèr, F. W., 171, 458, 459
Tarr Gloor, E., 213
Tarver, H., 121
Tatum, E. L., 24, 33, 40, 59, 79, 197, 206, 463
Tauber, H., 459
Taya, O., 171, 172
Taylor, A., 396
Taylor, C. B., 107
Taylor, C. E., 364, 478, 494
Taylor, D. J., 280
Taylor, E. P., 170
Taylor, H. E., 2
Taylor, H. F., 465
Taylor, M., 400
Taylor, P. W., 372
Taylor, R. M., 298, 312, 318 324
Tchan, Y. T., 25
Teas, H. J., 57
Teeters, C. M., 172
Teh-Cheng Huang, 267
Teichmann, E., 108
Temple, K. C., 109
Temple, K. L., 106, 107, 111
Tenenbaum, E., 497
Tener, G. M., 171
Tenhagen, M., 48
Tepper, B., 294
Terplan, K., 262
Terra, P., 214, 215
Terrill, R. J., 298
Terrill, S. W., 267
Terry, L. L., 294
Tetrault, P. A., 463
Thaler, H., 296
Thanassi, F. Z., 218
Thannhauser, S. J., 14
Thatcher, F. S., 449-472
Thatcher, J. S., 285
Thaysen, A. C., 462
Thaysen, J. H., 374
Thaysen, M., 462
Theile, E., 450, 451
Theiler, M., 298
Thiel, C. C., 430
Thiel, P. H., van, 133, 134, 140, 141, 145
Thofern, E., 183, 198
Thomas, B. F., 433
Thomas, E. L., 440
Thomas, J. B., 117, 123, 124, 125
Thomas, L. J., 293
Thomas, M., 107, 109
Thomas, R., 85
Thomas, R. C., 59, 97, 430
Thomas, S. B., 433
Thompson, J. L., Jr., 298
Thompson, P. E., 162, 283
Thompson, R., 370, 371
Thompson, R. L., 378
Thomson, D. R., 295
Thorling, L., 293
Thurston, J. P., 156, 274
Tidwell, W. L., 266
Tijn, B. van, 215
Tilden, E. B., 367
Timiras, P. S., 382
Timmens, E. K., 171
Tishkoff, G. H., 337
Tissier, M., 142
Tissières, A., 11, 81, 183, 184, 189, 190, 196
Tobias, C. A., 218, 222
Tobie, E. J., 284
Todd, E. W., 413
Tokuda, S., 117
Tolbert, N. E., 120
Tom, J. Y. S., 170, 261
Tomarelli, R. M., 63, 456
Tomcsik, J., 2, 3
Tome, J., 167
Tomiyasu, Y., 462
Tomlinson, T. H., Jr., 161
Tompsett, R., 385
Tones Muñoz, N., 299
Tonge, J. I., 146
Tonkin, I. M., 160
Toolan, H., 404
Toompas, C. A., 382
Topacio, T., 489
Torriani, A. M., 72, 196, 197
Tóth, J., 368
Tove, S. R., 121
Townsend, G. F., 190
Toyoda, J., 92
Tozawa, T., 458
Trager, W., 273, 275
Trano, Y., 57
Trapido, H., 299
Traub, E., 486
Traufler, D. H., 459
Treffers, H. P., 337
Trelawny, G. S., 114
Tria, E., 63
Troescher, C. B., 50
Tronnier, E. A., 13, 24, 25, 27
Tropeano, L., 360
Trout, G. M., 429
Trueta, J., 278, 284
Trumbull, M. L., 296
Tscherikower, R. S., 140
Tsou, K. C., 294
Tsuchida, M., 97
Tsuchiya, H. M., 85
Tsuchiya, T., 290, 298
Tsukamoto, A., 118, 119
Tsumargari, Y., 294
Tulasne, R., 23, 25, 128, 206
Turian, G., 122
Turner, A. W , 105
Turner, J., 394, 396, 401
Turner, L., 296
Twaddell, D. N., 295

U

Uchida, H., 6
Uchida, M., 91
Udenfriend, S., 77
Uhlenhuth, P., 134, 142, 143, 147
Uhley, C. G., 374
Ukita, C., 458
Ulebekova, M. V., 109
Umbarger, H. E., 58, 182, 183
Umbreit, W. W., 167-180; 81, 94, 105, 112, 167, 168, 170
Umezawa, H., 171, 172
Ungar, H., 144
Upward, M. G., 295
Utiger, H., 210
Utter, M. F., 87
Utz, J. P., 366, 369

V

Vago, G., 135
Vaisman, A., 169
Vajda, J., 368, 382
Valentine, W. N., 383
Vance, J. E., 49
Van den Berghe, L., 163
Van der Have, A. J., 429
Van Der Meer, S., 170
Vanderwerff, H., 62, 187
Van der Zant, W. C., 441
Van Heyningen, W. E., 199
Van Kesteren, M., 371
Van Lanen, J. M., 458
Van Orden, L. S., 169
Van Riel, J., 163
Van Rooyen, C. E., 488
Van Wagtendonk, W. J., 64, 215
Van Wijnagaarden, L. J., 262
Vaucel, M., 137
Vaughn, R. E., 79
Veen, J. van der, 312, 313, 314, 324
Veldstra, H., 170
Velick, S. F., 77
Veltre, F. A., 63

Vely, V., 11
Vendrely, R., 17, 24, 25, 206
Vennesland, B., 75
Verhoeven, W., 113
Vermeulen, D., 122
Vernon, L. P., 125
Viala, C., 397
Vicher, E. E., 259, 261, 262
Victor, J., 382
Vieting, G., 294
Vila, O. A., 171
Villela, G. G., 51
Vincent, E., 145
Vincke, I. H., 162, 163
Vinson, L. J., 451, 452, 454
Viollier, G., 169
Virgona, A., 167
Vischer, E. B., 172
Visconti, N., 201, 202, 251
Visconti di Modrone, N., 39
Vishniac, W., 110, 111, 112
Vivoli, D., 141
Vogel, F. S., 384
Vogel, H. J., 54, 58, 95, 96, 182, 195, 197
Vogesland, T. M., 216
Vogler, K. G., 112
Vogt, A. B., 188
Vogt, M., 223, 478, 482
Vojnovich, C., 459
Volcani, B. E., 59, 61, 107, 109
Volk, W. A., 122
Volkin, E., 16
Vollmer, E., 292
Vorhaus, L. J., 294

W

Waddington, H., 415, 422
Wade, G. R., 297, 298
Waelsch, H., 47, 57, 96
Wagener, G., 143
Wagenknecht, A. C., 121
Wagner, J. C., 368
Wagner, R. B., 167
Wagner, R. H., 81
Wagner, R. L., Jr., 171
Wagner, R. P., 58, 97, 183
Wahl, I., 463
Wahl, R., 200
Wainwright, J., 293
Wainwright, S. D., 219, 220
Waisbren, B. A., 257, 264
Wakaki, S., 172
Waksman, S. A., 168, 171, 172
Walch-Sorgdrager, B., 142
Waletzky, E., 273
Walker, J. B., 49
Walker, N., 91
Walker, T. K., 450
Wall, J. C., 51
Wall, J. S., 121
Wall, W. J., 163
Wallace, H. D., 267
Wallace, K. R., 413, 414
Wallace, U., 415, 422
Waller, C. W., 172
Wallgren, A., 293
Wallis, C., 364
Walshe, J. M., 294
Walt, J. P. van der, 6
Walter, A. W., 349
Walter, H., 347
Wang, C. H., 59, 97
Wanklyn, J. N., 109
Ward, C., 290
Ward, C. S., Jr., 291, 295
Ward, E. N., 481
Ward, H. K., 368
Ward, T. G., 476, 484
Ward, W. E., 296
Waring, G. B., 52
Warner, A. R., Jr., 140
Warner, L., 381
Warrack, G. H., 411, 413, 421
Warren, A. J., 298
Warren, K. B., 181, 199, 217
Warren, M. E., 411, 413, 421
Warren, W. R., 292
Wasserman, L., 458
Wasserman, M. M., 202
Wassink, E. C., 120, 122, 126
Wastler, T. A., 465
Watanabe, R. K., 297
Watrous, G. H., Jr., 440
Watrous, R. M., 379
Watson, B., 494
Watson, C. J., 294
Watson, D. W., 375, 377, 381
Watson, J. D., 181, 200, 201, 207, 217, 242, 243, 251
Watson, M., 217
Watson, P. L., 52, 186
Watson, R. W., 82
Weathersby, A. B., 161
Weaver, J. M., 62
Webb, M., 9, 49, 371
Webb, R. B., 7, 25, 33, 39
Webber, L., 400
Weber, M. M., 64
Wedekind, P., 292
Weed, L., 244
Weed, L. L., 245
Wegrich, O. G., 464, 465
Weibull, C., 6, 8, 13, 25
Weidel, W., 7, 200, 240
Weigl, J. W., 122, 125
Weigle, J. J., 181, 194, 221, 223, 249, 252
Weigmann, F., 370
Weihe, K. von, 167
Weil, A. J., 257, 260
Weingarten, B., 295
Weingarten, M., 294
Weinhouse, S., 79, 96, 451
Weinman, D., 136
Weinstein, M. J., 169
Weir, I. B. L., 142
Weiser, R. S., 370
Weisiger, J. R., 171
Weiss, A., 295
Weiss, E., 384
Weiss, U., 59, 92, 182
Weitkamp, A. W., 374
Welch, A. D., 52, 53
Welch, H., 170
Weller, S. D. V., 294
Weller, T. H., 473, 476, 477, 480, 482, 488, 489, 491, 492, 495
Wellington, N. A. M., 144
Wells, A. F., 465
Welsch, M., 25, 203
Welshimer, H. J., 7, 25
Wenner, H. A., 481
Wenrich, D. H., 26
Wenyon, C. E. M., 282
Werkman, C. H., 77, 96, 98
Werner, A. S., 116
Werner, G. H., 263
Werner, J. H., 313, 476, 484
Westergaard, M., 221
Westheimer, F. H., 75
Westwood, J. C. N., 312
Wetmore, P. W., 135, 140, 144
Whalen, W. M., 265, 266
Wharton, D. R., 355
Wharton, G. K., 295
Wheatley, A. H. M., 61
Whipple, A., 344
Whipple, G. H., 337
Whitaker, D. R., 462, 465
White, A. H., 442
White, E. G., 266
White, F. H., Jr., 94
White, H. R., 433
White, J., 452, 454, 464
White, L., 405
Whitehead, H. R., 438, 439
Whitehill, A. R., 48, 265
Whiteley, H. R., 87
Whitfield, J. F., 200, 246
Whitlock, C. M., Jr., 295
Whitman, A. L., 171
Whitman, L., 298
Whitmore, L. M., 167
Whitney, D. M., 375
Whittier, E. O., 458
Wiame, J. M., 14
Wickerham, L. J., 458
Widra, A., 39
Wielenga-Bergman, A. B., 143
Wieringa, K. T., 109
Wiesen, C. F., 458
Wiesendanger, S., 96
Wiesmann, E., 133, 134, 135, 136, 138, 139, 140, 144, 145, 146
Wight, K. M., 109
Wijesundera, S., 57
Wijngaarden, L. J., 188
Wikerhauser, T., 144
Wilcox, A., 161
Wilcox, C., 168, 170, 188, 259, 262
Wilcox, F. P., 430
Wildman, S. G., 16, 185
Wiley, A. J., 450, 452, 461, 462

Wilfuhr, G., 296
Wilhelm, R. H., 464
Wilkins, M. H. F., 217
Wilkinson, J. F., 3
Wilkinson, R. G., 170
Williams, D., Jr., 157
Williams, H. W., 425
Williams, I., 201, 247
Williams, J. H., 48, 49, 170
Williams, J. W., 172
Williams, O. B., 47, 56
Williams, R. C., 6, 199, 217, 237, 238
Williams, R. J., 56
Williams, V. R., 64
Williams, W. L., 267, 458
Williamson, J., 156, 158
Williamson, K. I., 438
Williamson, M., 189
Williams-Smith, H., 249
Willoughby, D. S., 440
Wilson, A., 294
Wilson, A. T., 373, 386
Wilson, C., 291, 292
Wilson, D. G., 109
Wilson, E., 114
Wilson, H. R., 217
Wilson, J. B., 115
Wilson, J. C., 481
Wilson, J. E., 266
Wilson, P. W., 92, 93, 115, 121, 184
Wilson, T. S., 142
Wiltshire, G. H., 91
Winge, O., 198, 211
Winje, M. E., 267
Winkler, A., 10, 15, 24, 27
Winogradsky, H., 106, 117
Winogradsky, S., 106
Winter, M. W., 156
Winterscheid, L. C., 7, 10, 13
Wirth, D., 142
Wirth, J., 489
Wirtz, H., 143
Wiseman, R. F., 443
Wiseman, R. W., 488
Witkin, E., 220
Wittebolle, P., 137, 138
Wittler, R. G., 424
Wolcott, G. B., 157
Wold, F., 54
Wolff, J. W., 136, 139, 141 142, 143, 144, 145
Wolfson, F., 156, 160
Wolfson, W. Q., 294
Wolinsky, E., 216, 261
Wollman, E. L., 72, 185, 203, 204, 205, 240, 249, 250
Womack, C. R., 474
Womack, M., 49
Wong, E., 383
Wong, S. C., 171
Wong, Y. T., 275, 279, 285
Wood, H. F., 294, 382
Wood, H. G., 88
Wood, R. K. S., 210
Wood, S., 48
Wood, T. H., 195, 218, 221
Wood, W. A., 54, 77, 78, 441, 442
Wood, W. B., Jr., 380, 382
Woodbine, M., 450
Woodruff, H. B., 48, 50
Woods, D. D., 56, 57
Woodward, K. T., 56, 169
Wooley, D. D., 65
Wooley, J. G., 251
Woolley, D. W., 452, 455
Woratz, H., 134, 136
Work, E., 115, 182, 195, 456, 457
Work, T. S., 167
Workman, W. G., 298
Wormall, A., 334, 337, 346
Worrel, C. S., 170
Wright, A. W., 297, 298
Wright, B. E., 193, 222
Wright, G. P., 346, 413
Wright, L. D., 62
Wright, L. T., 264, 265
Wright, M. H., 56
Wright, N. G., 95
Wright, S. S., 168, 170, 188, 259, 262
Wüstenberg, J., 443
Wyatt, G. R., 217, 242
Wyckoff, R., 25
Wyckoff, R. W. G., 237,251,374
Wylie, I. A. H., 145
Wynne, E. S., 168
Wyss, O., 167, 168, 170, 181, 187, 194, 219, 222, 223, 252

Y

Yager, R. H., 134, 135, 140, 144, 146, 477
Yamada, T., 92
Yamagata, S., 115, 146
Yamamoto, S., 143
Yamataka, A., 14
Yanamura, H. Y., 487
Yaniv, H., 169, 187, 188,216
Yanofsky, C., 95, 198, 251
Yaoi, H., 487
Yasowitz, H., 266
Yasukochi, H., 290, 298
Yates, M., 378
Ycas, M., 81, 183
Yocum, C. S., 120
Yoeli, M., 163, 375
Yokoyama, Y., 453
Yoneda, M., 56, 169
York, C. J., 144
Yoshida, A., 14
Yoshida, N., 135
Yoshimura, B., 283
Young, I., 300
Young, M. D., 157
Young, R. W., 295
Youngner, J. S., 481, 482
Yudkin, J., 185
Yuile, C. L., 337
Yuwaguchi, T., 172

Z

Zaharija, I., 143, 144
Zakrzewski, S. F., 52, 53
Zalokar, M., 184
Zamenhof, S., 115, 209, 217
Zamir, R., 267
Zanes, R., 405
Zanussi, C., 141
Zapf, K., 28, 38
Zatman, L. J., 75, 169
Zebovitz, E., 168
Zechmeister, L., 122
Zehren, V. L., 438
Zeigler, H. F., 455
Zeitlin, R. A., 296, 297
Zelle, M. R., 206
Zeller, E. A., 169
Zenitani, B., 462
Zickler, H., 212
Ziegler, A W., 210
Ziegler, D. W., 170
Zieve, B., 293
Zieve, L., 293
Zimmerman, H. J., 293
Zimmermann, P., 142, 143
Zinder, N. D., 2, 40, 186, 198, 208, 252
Zinsser, H., 368
Zirkle, R. E., 218
Zironi, A., 136
ZoBell, C. E., 105, 107, 109, 113
Zook, H. D., 167
Zschacke, J., 285
Zuckerman, A., 375
Zuckermann, A., 160
Zuelzer, M., 134
Zwartouw, H. T., 382
Zwemer, R. L., 285

SUBJECT INDEX

A

Acetate oxidation, vitamin B_{12} stimulation of, 50
Acetate-acetyl CoA conversion, 90
Acetic acid bacteria, oxidations by, 79
Acetobacter suboxydans stimulatory factor, 55
Acetyl transfer, 93-94
Acetylation, 93-94
Aconitase, 81
Acridines, "petite" induction by, 190-91
Actithiazic acid, 171
Acute respiratory disease viruses, cellular degeneration by, 476
Adaptive enzyme formation, 71-74
  scheme for, 74
  and variation, 194
  amino acid requirements for, 73-75
  influence of phage on, 72
  and 2-ketogluconate oxidation, 78
Adaptation
  and age of cells, 463
  auto-, 193
  industrial applications of, 463
  long term, 191
  simultaneous, 91
Adenine, metabolism of, 62
Adenoid degeneration agents, cell inclusions and degeneration by, 484
Adenylic acid, and triphosphopyridine nucleotide oxidation, 75
Adsorption of phages, 239-40
Agglutination, relation of flagella to, 6
Agglutination-lysis test, in leptospiroses, 139-41
Agglutinins, cold, relationship of polysaccharides to, 360
Agricultural wastes, utilization of, 462-63
Alcohols, secondary, oxidation of, 118
Aldehyde dehydrogenase, 90
Algae, genetics of, 215
Amides, magnesium and biosynthesis of, 49
Amino acid antagonisms, 58-59
Amino acid decarboxylase, 94-95
Amino acids
  and adaptive enzyme formation, 73-75
  biosynthesis of, 57-60
  complex interactions in biosynthesis of, 183
  genetic analysis of metabolism of, 182-83
  incorporation of, 94
  inhibitions by, 183
  metabolism of, 93-97
  nutritional requirements for, 56-60
  and photosynthesis, 119
  as precursors in enzyme synthesis, 196-97
  requirements for
    and carbon source, 58
    and virulence, 183
  utilization of, review, 96
Ammonia, oxidation of, 92, 113
Amoebiasis, 280-83
  experimental extraintestinal, 283
  extraintestinal, 281-82
  hepatic, 282
  intestinal, 280-81
    invasion in, 280-81
Animal nutrition, use of antibiotics in, 265-67
Antibacterial action, 167-80
Antibiotic activity, metabolite reversal of, 65
Antibiotic resistant strains, incidence of, 258-61
Antibiotics
  in animal nutrition, 265-67
  combinations of, 261
  cross-resistance between, and mode of action, 65
  effects on Leptospira, 145, 147
  influence of, upon secondary flora, 262-65
  and microbial nutrition, 64-65
  miscellaneous, structures of, 172
  mode of action of, reviews on, 167
  and nutrition, 458
  as prophylactic agents in surgery, 264-65
  resistance to, 258-62
  techniques for study of, 173
  types of interactions of, 261-62
Antibiotic therapy, and resistance to, 258-62
Antibodies
  adsorption of
    and influenza antigens, 314
  duration of production of, 349
  fluorescent, 343-45
  formation of
    and persistence of antigen, 347-49
  iodine 131 labelled, 334-36
  labelled, 333-52
Antibody response, and antigen elimination, 340-41
Antigen-antibody reactions, labels for, 333-37
Antigenic components of bacteria, location of, 7
Antigenic sites on flagella, 6
Antigenic variation of influenza viruses, 311-32
Antigens
  azoprotein labelled, 336-37
  cellular deposition of, 342-43
  elimination of
    and antibody response, 340-41
  Forssman
    in animal parasites, 358
  histochemical methods for tracing, 337
  of influenza viruses, 314
  labelled, 333-52
    with C^{14}, 337
    with I^{131}, 334-36
    with N^{15}, 337
    with P^{32}, 337
  leptospiral, 136-41
  persistence of, and antibody formation, 347-49
  polysaccharides as, 354-55
  radio-labelled
    localization of, 345-47
  soluble bacterial, 412
    discriminating methods for, 412-24
  temperature sensitive, 192-93
  as templates, 349
  variation of, 192-93
Antimicrobial factors, in tissue extracts, 375-77
Antitoxins, loss of, from circulation, 338
Antiviral agents, 173
Arabinose, adaptive enzyme for, 83
Arginine desmidase, 96
Aromatic acids, biosynthesis of, 182

Aromatic ring, biosynthesis of, 59-60
Ascites tumors of mice, effect of viruses on, 401-3
Ascomycetes, genetics of, 212
Aspartic acid
  metabolism of, 95
  utilization of, 95
Aureomycin (Chlortetracyclene)
  and nitro group reduction, 76
  see also Tetracyclenes
Auto-adaptation, 193
Avain pest virus, effect of, on tumors, 393
Azoproteins
  as antigens, 336-37
  cellular deposition of, 342-45

B

Bacteria
  antigenic structure of, 192-93
  cytology of, 1-21, 23-46
  as food source, 459-60
  genetics of, 206-9
  life cycles of, 206
  miscellaneous
    and milk pasteurization, 432-33
  osmotic barrier of, 8-10
  particulate enzymatically-active particles from, 11
  pathogenic, and milk pasteurization, 429-32
  protoplasts of, 8-9
  psychrophilic, in dairy products, 440-41
  sexuality in, 40
  thermoduric, in milk, 433-34
Bacterial cell
  phage-induced
    chemical changes in, 246-47
    enzymic changes in, 246-47
Bacterial filtrates
  discriminating methods for, 412-24
    dilution, 412-14
    immunological, 414-16
    serological, 416-24
  observable activities of, 412
Bacterial growth, influence of tissue metabolities on, 377-78
Bacterial nucleus, 23-46
  basic concepts of, 23-24
  methods for demonstration of, 24-28
  phage-induced changes in, 246
  summary of evidence for, 41-42
Bacterial spores, nuclei of, 40-41
Bacterial structures, microphotographs of, 4
Bacterial toxins, 411-28
Bacterial viruses
  see Bacteriophage
Bacteriochlorophyll, types of, 125
Bacteriocins, 205
Bacteriophage, 237-56
  adsorption of
    by bacterial membranes, 240
  coli, action of Shigella antigen on, 240
  conversion of temperate to virulent, 248-51
  effect of, on host metabolism, 201-2
  fate of constituents of, 240-41
  formation of, 241-51
  gene-like nature of, 249
  general studies on, 251-52
  genetic interactions between, 204-5
  genetic recombination in, 251-52
  host-induced modifications in, 194
  and hosts, genetic interactions between, 204-5
  influence of, on toxicity of diphtheria bacillus, 252
  invasion by, 200
  lysogenic, physiology of development of, 205
  maturation of, 250
  morphology of, 199-200
  new species of, 239
  nutritional factors influencing formation of, 250-51
  origin of DNA in, 200-1
  origin of protein in, 201
  phenotypic variations in, 194
  primary reversible adsorption of, 239
  receptors of, 7
  recombination in, 202-3
  release of, by premature lysis, 242
  result of superinfection with, 241
  secondary irreversible attachment of, 239-40
  source of constituents of, 242-51
  splitting of particles of, 239-40
  and starter culture defects, 437-39
  T-7, origin of P and N of, 245
  T-even
    diagram of, 238
    origin of C, N, and P of, 244
  T series, 237-38
    antigenic properties of, 238
    composition of, 237
    morphology of, 237
  taxonomy of, 199
  temperate, 247-51
  temperate and virulent, comparison of effects of, 250-51
  temperature and activity of, 248
  virulent, 239-47
  resistance, mutation to, 185
Barrage character, and growth pattern, 191-92
Basidiomycetes, genetics of, 214-15
Benzoic acid
  adaptive enzymes for, 74
  oxidation of, 74, 91
"Biochemical lesions" in tissue cultures, 474
Biosynthesis
  and gene action, 196-97
  of penicillin, 167
  see also specific compounds
Biotin
  as growth factor, 56
  interrelationship with pantothenic acid, 54
  substitutes, 56
Blood, disappearance of proteins from, 337-42
Blood serum, and host defenses, 365-69
Brucella abortus, transmission of, in milk, 431
"Buller phenomenon," 214-15
Bunyamwera virus
  effect of
    on mouse ascites tumors, 402
    on tumors in man, 404-5

C

Calcium, influence of, on bacterial nutrition, 49
Canicola infection, 141
Canine leptospirosis, 142-43
Capsules
  demonstration of, 3
  nature of, 2-3
  significance of, 3
Carbohydrates, in fungi, 457
Carbomycin, 171
Carbon dioxide assimilation

by chemoautotrophs, 112
efficiency of, 115
fixation, 84
photosynthetic assimilation of, 120
tension
nutritional requirements of bacteria, 48
Carbon monoxide oxidation, 115
Carotenes, in fungi, 458
Cell wall
of bacteria, 7-8
demonstration of, 7
and nuclei of bacteria photomicrographs of, 30
and nucleus
simultaneous demonstration of, 27
separation of, from protoplast, 7-8
ultramicroscopic structure of, 7-8
Cellular degeneration, virus induced, 476-83
Cellular deposition of antigens, 342-43
Cellular poisons, and production of infection, 385
Chemoautotrophic bacteria, 105-16
biochemistry of, 111-16
chemical analyses of, 115-16
culture methods for, 107-9
distribution of, 106
efficiency of assimilation by, 115
inhibitory action of organic compounds on, 111-12
isolation of, 107-9
physiology of, 111-16
role of, in nature, 109-11
taxonomy of, 106-7
types of, 105
Chemotaxis, and phagocytic activity, 380-81
Chemotherapeutic agents
and host flora, 257-72
see also Antibiotics; and specific agents
Chemotherapy
enterocolitis following, 362-63
miscellaneous infections following, 264
moniliasis following, 263-64
and secondary flora, 262-65
Chain formation, in bacteria, influence of magnesium on, 49
Chemical nature of flagella, 6
Chloramphenicol, action of, 169-70
Chromatophores, 117
of photosynthetic bacteria, 16
Chromosome behavior, during spore germination, 41
Chromosomes, in bacterial nucleus, 35-36
Citrate oxidation factor, 81
Citric acid cycle
see Tricarboxylic acid cycle
Citric acid dissimilation, 80
Citric acid oxidation, new pathway for, 80-81
Citrovorum factor, 53-54
Citrulline-ornithine reaction, 96
Coenzyme A, and propionate formation, 87
Coenzyme A transphorase, 90-91
Coenzyme III, 97
Colchicine, effect of, on bacterial nucleus, 36, 38-39
Cold agglutinins, relationship to polysaccharides, 360
Complement, and host defenses, 366-68
Copper requirements of microorganisms, 49
Coprogen, 48
Coxsackie virus
cellular degeneration by, 476-77
effect of, on tumors, 393-94
Cross-sensitization, relation of polysaccharides to, 357
Cultures, preservation of, 463
Cyanocobalamin
see Vitamin B_{12}
Cyclitols, oxidation of, 83
Cysteinesulfinic acid, metabolism of, 97
Cytochrome
inactivating systems for, 81
in photosynthetic bacteria, 125-26
Cytochrome-c, 76
Cytology of bacteria, 1-21, 23-46
Cytopathic
see Cytopathogenicity
Cytopathogenic effects, types of, in tissue cultures, 474
Cytopathogenicity
definition of, 473-74
viral
morphological manifestations of, 475-93
of viruses
general comment, 497-98
Cytopathology of virus infections, 473-502
Cytoplasmic membranes
of bacteria, 8-9
selective staining of, 9
Cytoplasm-nucleus, interrelations between, 192
Cytoplasmic autonomy, 193
Cytoplasmic factors, and variation, 188-92
Cytoplasmic inheritance, and phenotypic variation, 188-95

D

Dairy products
enzymes in, 441-42
media for testing of, 442-44
methods for testing of, 442-44
microbiology of, 429-48
psychrophilic bacteria in, 440-41
Dairy wastes, utilization of, 460-61
Decarboxylase, amino acid, 94-95
Decarboxylation, 86-87
and lipothiamide phrophosphate, 86
"Decision," in phage infection, 204
Dengue, 300-2
general studies of, 300-1
host range of, 301
immunity against, 301-2
properties of virus of, 301
relationship to other viruses, 302
virus, effect of, on tumors in man, 405
Disoxyribonucleic acid
see DNA
Dextran synthesis, 85
Diaminophimelic acid, 115
Diauxie, 71-72
Diptheria bacteria, phage induction of toxicity in, 252
DNA
and genetic, 216-17
structure of, 217-18
Drugs, growth dependence upon, 187
Drug resistance, 185-88
biochemical mechanisms of, 186-87
genetic control of, 185-86
phenotypic effects and, 187-88

E

Eastern equine encephalitis virus, effect of, in tissue cultures, 403
Ectromelia virus, cell inclusions and degeneration by, 484

Encephalitis viruses, effect of, on tumors, 398-99
Endemic influenza, 324-26
Endospores, electron microscope studies of, 8
Enterocolitis, 262-63
Environment, and radiation effects, 219-221
Enzymes
  adaptive, formation of, 71-74
  biosynthesis of, and gene action, 196-97
  in dairy products, 441-42
  in food industry, 459
  and gene action, 195-96
  of phagocytes, 383-86
  serological cross reactions of, 72
  of serum, and body defenses, 369
Epidemic exanthem, viruses from, and cellular degeneration, 483
Epidemic influenza, 324-26
Equine abortion virus, cell inclusions and degeneration, 485
Equine encephalomyelitis viruses, cellular degeneration by, 477-78
Ergosterol, in fungi, 457-58
Erythromycin, 170
Evolution, and population genetics, 216
Exo-erythrocytic schizogony, in malaria parasites, 160-61

F

Fats, microbial production of, 449-51
Fatty acids
  biosynthesis of, 184
  oxidation of, by purple bacteria, 118-19
  sparing action on biotin, 56
Fecal viruses, undifferentiated, cellular degeneration by, 482-83
Feline panleukopenia virus, effect of, on tumors in man, 405
Fermentation, 83-85
Fermentation industry, problems in, 463-64
Fermentations,
  influence of pH on, 464
  influence of temperature on, 464
Ferrichrome, 48
F factor, role of phage in, 251
Flagella, 3-7
  and bacterial agglutination, 6
  organelle nature of, 5
  structural differences between, 6
Flagellar determinants, transduction of, 6
Fluorescent antibody, 343-45
Folic acid
  see Pteroylglutamic acid
Folinic acid
  see Citrovorum fact
Food
  from bacteria, 459-60
  enzymes in production of, 459
  from fungi, 449-72
    general aspects of, 464-66
  production, substrates for, 460-63
Foot and mouth virus
  cellular degeneration by, 478-79
  effect of, on tumors, 394
Formate
  and biosynthesis of citrovorum factor, 53
  as metabolic intermediate, 88
  metabolism, sulfathiazole inhibition of, 90
  schemes for decomposition of, 89-90
Formic dehydrogenase, 88
Forssman antigen, in animal parasites, 358
Fowl plague virus, cell inclusions and degeneration, 485-86
Fowl pox virus, cellular changes induced by, 493
Fructose transfer, 84-85
Fumarase, 81
Fungi
  carbohydrates in, 457
  carotenes in, 458
  ergosterol in, 457-58
  extra-sexual recombination in, 212-14
  foods and feeds from, 449-72
  genetics of, 210-15
  nucleic acids in, 459
  nutritional value of, 454-57
  riboflavin in, 458-59
  vitamin B_{12} in, 458

G

Galactokinase, 84
β-Galactosidase
  formation, inducers of, 73
  induction of, 72
  synthesis of protein of, 72-73
β-Galactozymase, formation of, 71-72
Gastrointestinal secretions, and body defences, 370-72
Gene action, 195-99
  and biosynthesis, 196-97
  and enzymatic constitution, 195-96
  theory of, 198-99
Genetic analysis, of intermediate metabolism, 182-84
Genetic determination of flagellation, 6
Genetic material
  nature of, 216-23
  properties of, 216-23
  spontaneous change of, 218-23
Genetic parameters, 221
Genetics
  of algae, 215
  and amino acid metabolism, 182-83
  of ascomycetes, 212
  bacterial, 206-9
  of basidiomycetes, 214-15
  books on, 181
  and cytoplasmic factors, 188-92
  of drug resistance, 184-85
  of fungi, 210-15
  haploid, and tetrad analysis, 209-10
  methods of, 223-24
  of microorganisms, 181-236
  population, and evolution, 216
  of protozoa, 215-16
  reviews on, 182
  terminological complexities in, 181
  transmission, and life cycles, 199-216
Genito-urinary secretions, and body defenses, 372-73
Glucose
  B. subtilis fermentation of, 83-84
  oxidative pathways for, 77-78
  phosphate, pathway of oxidation of, 78
  Rhizopus fermentation of, 84
  transfer, 85
Glucose-6-phosphate dehydrogenase, 79-80
Glutathione, biosynthesis of, 94
Glyceraldehyde-3-phosphate dehydrogenase, 76-77
Glycerol oxidation, 83
Glycine, need for, in synthesis, 82
Gram stain, role of cytoplasmic membrane in, 9
Grana, 117

Growth factors
for chemoautotrophs, 109
unknown, 63-64

H

Hemagglutinating viruses, effect of, on mouse ascites tumors, 401-2
Hemoglobin in microorganisms, 82
Hepatitis
chronic, 293
laboratory studies of, 293-94
prevention of, 295
serus, 295-98
treatment of, 294-95
viral, 289-98
virus A, 289-95
Hepatitis virus, effect of, on tumors in man, 405
Herpes simplex virus
cellular changes induced by, 490-91
effect of
in tissue cultures, 403
on tumors, 394
Herpes zoster virus, cellular changes induced by, 491-92
Heterocaryosis, 214-15
Heterothallism, 206-8
Hexosemonophosphate shunt, 79-80
Histidine
biosynthesis of, 182
decarboxylase, 96
Histochemical methods, for tracing antigens, 337
Hormones, biosynthesis of, 183-84
Host
humoral defenses of, 365-69
and virus-tumor relationships, 407-8
Host flora, influence of chemotherapeutic agents upon, 257-72
Host-phage relationships, 239-51
Host resistance, tissue extracts in study of, 374-78
Humoral defenses of host, 365-69
Hydrogen, enzymatic transfer of, 74-75
Hydrogen bacteria, and utilization of organic compounds, 114
Hydrogenase, 113-14
and nitrogen fixation, 93
Hydrogenlyase, 88
Hydrogen
oxidation of, 107
and nitrate reduction, 114
photochemical evolution of, 121
Hydroxylamine reductase, 92
5-Hydroxymethylcytosine, 242
Hypersensitivity, relation of polysaccharides to, 357

I

Ilhéus virus
effect of
on mouse leukemias, 400
on tumors in man, 405
Ilotycin, 170
Imidazole compounds, and acetyl transfer, 93
Immunity
acquired, role of polysaccharides in, 356
nonspecific factors in, 363-92
Immunization, against influenza, 326-28
Immunological properties, of animal parasite polysaccharides, 353-62
Immunology, use of isotopes in, 337
Inclusion bodies
and cell degeneration, viruses causing, 483-90
formation of, in tissue cultures, 474
Indole, oxidation of, 91
Infections, virus, cytopathology of, 473-502
Infectious canine hepatitis virus, cell inclusions and degeneration, 486
Infectious hepatitis
clinical course of, 292-93
epidemiology of, 290-92
hepatitis virus A, 289-90
Influenza
antigenic variation and immunization, 327-28
effect of, in tissue culture, 403-4
endemic, 324-26
epidemic, 324-26
pandemic, 322-24
prophylactic immunization against, 326-28
Influenza A
antigenic variations of, 312-15
PQR variation of, 314-15
subgroups of, 313
Influenza B, 315
Influenza C, 315
Influenza viruses
antigenic variation of, 311-32
cellular changes induced by, 493-94
effect of
on mouse ascites tumors, 402
on tumors, 394
immunologic selection of, 318
mechanisms for recombination of, 320-22
mechanisms for variation of, 315-22
mutation of, 315-17
reactivation of, 321
recombination of, 318-20
significance of variation of, 322-28
variation of, 317-18
in nature, 311-15
Iodine 131, as label in antigen-antibody reactions, 334-36
Iron bacteria, survey of, 107
Iron corrosion, 109
Isocitric acid dehydrogenase, 81
Isoniazid
competitive inhibition of, by vitamin B_6, 56
growth dependence upon, 187
neutralization of toxicity of, by biotin, 56
Isotopes, use of, in immunology, 337

K

Kappa, 189
Keto acid decarboxylation, 86-87
2-Ketogluconate, adaptive enzymes for, 78
α-Ketoglutaric oxidase, 81
Kojic acid, formation of, 80

L

Labelled antibodies, 333-52
Labelled antigens, 333-52
Latent viruses
effect of
on mouse leukemias, 400-1
on tumors, 394
Leptospira, 133-52
antigenic properties of, 136-41
distribution of, 145-46
effect of antibiotics on, 145, 147
growth requirements of, 135-36
immunization against, 145
metabolism of, 135-36
morphology of, 134-35
systematic position of, 133-34
types of, 138-41
Leptospiroses
miscellaneous, 143-45

serological disgnosis of, 136-41
serum therapy in, 145
canine, 142-43
Leucine, incorporation of, in proteins, 94
Leucovorin
see Citrovorum factor
Leukemias, mouse, effect of viruses on, 400-1
Life cycles
of bacteria, 206
and transmission genetics, 199-216
Life history, of malaria parasites, 153-66
Lipids
antimicrobial activity of, 377
as possible growth factors, 64
in serum, and body defenses, 369
Lipoic acid
function of, 85-86
nature of, 86
Lipomucoproteins as somatic antigens and phage receptors, 7
Lipoporteins as cell wall compenents, 7
Lipothiamide pyrophosphate, and decarboxylation, 86
Lithium toxicity for bacteria, and pH, 49
Luminescence, factors essential for, 92
Lymphogranuloma virus, effect of, on tumors, 396-97
Lymphogranuloma inguinale virus, cell inclusions and degeneration, 486-87
Lysine, biosynthesis of, 96
Lysogenic bacteria
agents inducing active phage in, 249-50
morphological changes in, during phage multiplication, 250
Lysogeny, 203-5
characters associated with, 205
induction of, 203-4
nature of, 247-51
physiological aspects of, 205

M

Macromolecules, and microsomes, in bacteria, 15-17
Magnamycin, 171
Magnesium
and biosynthesis of amides, 49
influence of, on cellular division, 49
Malaria, 273-79
anaemia, anoxaemia, and tissue anoxia, 278
effect of
on blood of host, 273-77
on blood levels, 275
on blood lipids, 277
on blood pigments, 276
on blood protein, 276-77
on blood volume, 275-76
on erythrocytes, 273-75
general effects of, 277-79
host and parasite, 273
tissue changes in, 278-79
Malaria parasites
differential characters in tissue phase of, 158-60
gametocytes in blood, 154-55
life history of, 153-66
new species of, 162-63
reviews on, 153
schizonts and gametocytes in blood, 155-56
tissue stages of, 154-62
Maltozymase formation, inhibitors of, 72-73
Mammary gland secretions, and body defenses, 373
Mandelic acid, dissimilation of, 91
Mate-killing, 188-89
Mating types
determination of, 192
in yeast, 210-11
Maturation of phage, 250
Measles, agents from, cellular changes induced by, 492-93
Microbiology of dairy products, 429-48
Microorganisms, nutrition of, 47-70
Microsomes, and macromolecules, in bacteria, 15-17
Minerals, nutritional requirements for, 48-49
Mitochondria (reduktionsorte, crops métaboliques, metabolitkörper, chondrioides), 10-15
release of, 190
Mitochondria in bacteria
arguments against, 11-13
definition of, 11
demonstrations of, 10-11
Mitosis in bacteria
evidence against, 18-33
evidence for, 33-40
photomicrographs of, 30
Mitosis in the bacterial nucleus, 28-40
Mechanisms of antibacterial action, 167-80
Membranes, bacterial, adsorption of phage by, 240
Mengo encephalitis virus, effect of, on mouse ascites tumors, 403
Metabolities, influence of, on antibiotic activity, 65
Metabolism
intermediate, genetic analysis of, 182-84
of leptospiras, 135-36
of microorganisms, 71-104
oxidative, deficiencies in, 184
Metachromatic granules, 12-15
chemical nature of, 14-15
Metaphosphate, and metachromatic granules, 14-15
Methionine
biosynthesis of, 57-58, 182
metabolism of, 96-97
substitutes for, 56-58
Methionine sulfoxide
biological activity of, 56-57
toxicity of, 57
Methods
of genetics, 223-24
for testing dairy products, 442-44
Modifier and suppressor action, 197-98
Molasses, utilization of, 462
Molybdenum
and nitrate reductase, 49
requirements of microorganisms, 49
toxicity, reversal by vitamin B_{12}, 51
Moniliasis (candidiasis), 263-64
Morphological mutations, 184-85
Morphology
of Leptospira, 134-35
of phages, 199-200
Mouse leukemias, effect of viruses on, 400-1
Multinucleated cell formation, viruses inducing, 490-93
Multiple factors, and pseudoallelism, 198
Mumps virus, cellular changes induced by, 494
Mutagenesis, 222
Mutations
of influenza viruses, 315-17
morphological, 184-85
and physiological variation, 221
and plating density, 221
pleiotrophic, 196

N

Naphthalene, oxidation of, 91
Nasal secretions, and body defenses, 370
Neoplastic viruses, cellular changes induced by, 496-97
Neurotropic viruses
  effect of
    on mouse ascites tumors, 402-3
    on tumors, 394-95
Newcastle virus
  cellular degeneration by, 479-80
  effect of, on tumors in man, 405
Nitratase, 92
Nitrate reductase, influence of molybdenum on, 49
Nitrate reduction, 92
  hydrogen oxidation and, 114-15
Nitrification, 110
Nitritase, 92
Nitrogen fixation, 92-93
  by photosynthetic bacteria, 121
Nitrogen metabolism, 92-98
  enzymatic deficiencies in, 184
Nitro group reduction, 76
Nonspecific factors in immunity, 363-92
Nuclear behavior, during bacterial sporulation, 40-41
Nuclear membrane, in bacteria, evidence for, 36-37
Nucleic acids, in fungi, 459
Nucleus
  of bacteria, 23-46
    and divisional mechanism, 34
    effect of drugs on, 36-37
    electron microscopy of, 37-38
    phase microscopy of, 38
    postulated types of, 39
    summary of evidence for, 41-42
  bacterial
    phage-induced changes in, 246
  and cell walls
    of bacteria, photomicrographs of, 30
    simultaneous demonstration of, 27
  of endospores, 8
  interphase, 211
  of spores
    evidence against extracytoplasmic location of, 41
Nucleus-cytoplasm, interrelations between, 192
Nutrition, animal, use of antibiotics in, 265-67
Nutritional requirements of bacteria
  effect of anaerobiosis on, 48
  effect of oxygen on, 48
Nutrition of bacteria, and phage production, 250-51
Nutrition of microorganisms, 47-70

O

Oligosaccharide formation, 84-85
Ornithine, biosynthesis of, 95, 182
Ornithosis virus, effect of, on tumors, 397
Orotic acid
  degradation of, 62
  metabolism of, 98
Osmotic barrier
  of bacteria, 8-10
    thickness of, 8
  thickness of, 9
Osmotic shock, of phages, 238, 247
Oxidation-reduction potential
  and B_{12} requirement of bacteria, 48
  and biosynthesis of citrovorum factor, 53
  and pyrimidine requirement of bacteria, 48
Oxidation state, and radiation damage, 219
Oxidative metabolism, deficiencies in, 184
Oxidative pathways, 77-83
  relative activity in, 79
Oxygen tension, and nutritional requirements of bacteria, 48

P

Pandemic influenza, 322-24
Pantetheine, formation from pantothenic acid, 54-55
Pantoic acid, biosynthesis of, 54, 182
Pantothenic acid, 54-55
Parameters, genetic, 221
Parasites, animal, polysaccharides of, 353-62
Pasteurization, 429-34
Pathogens, destruction of, in milk, 429-32
Penicillin
  action of, 167
  biosynthesis of, 167
  combinations with other antibiotics, 168
  enhancement of action, 167-68
  growth dependence upon, 187
  production of, 167
Penicillinase formation, induction of, 73-74
Peptides
  activity of, compared to amino acids, 60-61
  physiological activities of, 377
  nutritional requirements for, 60-61
  toxic, 171-72
Peroxidation, 76
"Petites," 190-91
pH, and fermentation products, 464
Phage
  see Bacteriophage
Phagocytes
  and body defense, 378-80
  engulfment of pathogenic bacteria by, 381-83
  enzymology of, 383-86
Phagocytosis, 378-86
  and chemotaxis, 380-81
Phase microscopy, and demonstration of bacterial structures, 28
Phenol
  formation of tyrosine from, 91
  mutants resistant to, 194-95
Phenotypic delay, 223
Phenotypic effects, and drug resistance, 187-88
Phenotypic variation, and cytoplasmic inheritance, 188-95
Phenylalanine
  biosynthesis of, 59-60
  oxidation of, 97
Phosphogluconate, oxidation of, 78
6-Phosphogluconate dehydrogenase, 79
2-Phospho-4-hydroxy-4-carboxyadipic acid, 81
Phosphorus exchange, and bacterial carrier molecules, 8
Photochemical evolution of hydrogen, 121
Photoreactivation, 220-21
Photosynthesis
  bacterial quantum yield in, 120
  pigments, and phototaxis, 122-26
  primary photochemical reaction in, 125
Photosynthetic bacteria, 116-26
  biochemistry of, 118-121
  chromatophores in, 16

culture methods for, 116-18
distribution of, 116
inhibitory effect of glycine on, 121
light and dark metabolism of, 118
location of pigments in, 117
morphology of, 116-18
physiology of, 118-121
pigments and phototaxis in, 122-26
taxonomy of, 116-18
tricarboxylic acid cycle in, 119
Phototaxis, 116, 123-24
Phycomycetes, genetics of, 210
Physico-chemical factors influencing growth of bacteria, 47-48
Physiological aspects of protozoan infection, 273-88
Physiological variation, and mutation, 221
Pigment formation, variation in, 193
Pigments
biosynthesis of, 184
of photosynthetic bacteria, 122-26
Plant viruses, RNA in, 217
Plasmodium
avian species of
P. cathemerium, 161
P. fallax, 161
P. gallinaceum, 161
P. hexamerium, 162
P. huffi, 162
P. juxtanucleare, 161
P. lophurae, 161
P. vaughani, 162
definition of genus, 153
mammalian species of
P. cynomolgi, 156-57
P. falciparum, 157
P. inui, 157-58
P. ovale, 158
new species of, 162-63
saurian species of, 162
see also Malaria parasites
Pleiotrophic mutation, 196
"Poky," 190
Poliomyelitis
transmission of, in milk, 431-32
virus
cellular degeneration by, 480-82
effect of, in tissue culture, 404
Polypeptides, inhibitory effect of, on growth, 61
Polyploid state, of bacterial nucleus, 36-37, 40
Polysaccharides
and acquired immunity, 356
of animal parasites, summary of, 360-61
as complete antigens, 354-55
as diagnostic antigens, 355
immunological properties of, 353-62
methods of preparation of, 353-54
relationship to cross-sensitization, 357
relationship to hypersensitivity, 357
relationship to sheep cell antigens, 357-58
of worms, relationship to red blood cell antigens, 358
Population genetics and evolution, 216
Porphyrin, biosynthesis of, 183-84
Pox viruses, effect of, on tumors, 395-96
Precipitin tests, comparison of nitrogen and radioactivity in, 335
Proline, biosynthesis of, 95
Propionate, inhibition of metabolism by, 86
Propionic acid,formation of, 87-88
Prophage, 204, 248
conversion of, to active form, 248
and preprophage, 204
see also Lysogeny
Proteins
amino acids in, 456
disappearance of, from circulation, 337-42
food, species producing, 452
iodine 131 labelled, 334-36
labelled, synthesis of, 121
microbial production of, 451-54
microbial, toxicity of, 456-57
radio-labelled, localization of, 345-47
of viruses, 217
Protoplasts of bacteria, 8-9
Protozoa, genetics of, 215-16
Protozoan infections
general aspects of, 283-85
miscellaneous, 283-85
physiological aspects of, 273-88
Pseudoallelism, and multiple factors, 198
Pseudomonads, oxidative pathways in, 77-78
Pseudorabies virus
cell inclusions and degeneration, 486
effect of, in tissue culture, 404
Psittacosis virus, 396-97, 487
Psychrophilic bacteria, in dairy products, 440-41
Pteroylglutamic acid, 51-53
antagonists for, 52-53
and ornithine decarboxylase, 51
as precursor of citrovorum factor, 54
sparing effect of thymine on, 51
Pteroylglutamic acid antagonists, need for purity of, 52
Purine metabolism, 183
Purines
interconversion of, 62-63
metabolism of, 97-98
nutritional requirements for, 62-63
Pyridine nucleotides, and hydrogen transport, 75-76
Pyridoxal phosphate, pteroylglutamic acid and biosynthesis of, 51
Pyrimidine metabolism, 183
Pyrimidine requirement of bacteria, influence of O/R potential on, 48
Pyrimidines
metabolism of, 97-98
nutritional requirements for, 62-63
Pyruvate fermentation, by photosynthetic bacteria, 120-21
Pyruvic acid, metabolism of, 85-87

Q

Q fever, transmission of, in milk, 430
Quantum yield, in photosynthesis, 120

R

Rabies virus
cellular changes induced by, 494-95
effect of
on tumors, 397-98
on tumors in man, 405
Radiation
damage
and chemical effects, 219-20
and oxidation state, 219
and photoreactivation, 220
and temperature effects, 220
and ultraviolet reactivation, 221
and environmental effects, 219-21
killing effects of, 218-19

mutagenic effects of, 218-19
Raffinose fermentation, genetic control of, 211
Reactivation of influenza viruses, 321
Recombination
in animal viruses, 203
in bacteria, 206-8
in fungi
extra-sexual, 212-14
of influenza viruses, 318-20
in phages, 202-3
Renal tissues, agents from, cellular changes induced by, 493
Resistance
to antibiotics, incidence of, 258-61
and culture age, 221
to disease, general factors in, 364-65
phenotypic variation in, 195
Rhesus monkeys, renal tissues of, and cellular changes induced by, 493
Riboflavin, in fungi, 458-59
Ribonucleic acids, in yeast, differences between, 98
Ribulose-5-phosphate, dissimilation of, 83
Rickettsiae, effect of, on tumors, 398
Rickettsial pox, effect of, on tumors in man, 405
Rift Valley fever virus, cellular changes induced by, 495
Rous sarcoma virus, effect of, on tumors, 398
Russian encephalitis virus, effect of viruses on, 401
Russian far east encephalitis virus, effect of, on tumors, 399

S

St. Louis encephalitis virus
cellular changes induced by, 495
effect of, on mouse ascites tumors, 403
Salivary gland disease virus, cellular changes induced by, 495
Schizogony, exo-erythrocytic, in malaria parasites, 160-61
Sedoheptulose, transfer reactions from, 82
"Semi-incompatibility," 212
Semliki Forest virus, effect of, on tumors, 399
Serine, biosynthesis of, 95
Serine dehydrase, 95
Serum hepatitis, 295-98
epidemiology of, 297
prevention of, 297
transmission of, 295-96
virus B of, 296
Sexuality in bacteria, 40
Shikimic acid
and biosynthesis of amino acids, 59-60
formation of, 92
Silica gel, preparation of, 107
Simultaneous adaptation, 91
Skin and body defense, 373-74
Sodium para-aminosalicylate, effect of, on bacterial nucleus, 36
Soil perfusion technique, 109-10
Sparking systems, 88-89
Spermine, 376
Spinal fluid, and body defenses, 369-70
Spore germination, nuclear behavior during, 40-41
Sporulation of bacteria, nuclear behavior during, 40-41
Starter cultures, dairy, 434-40
bacteriophages in, 437-39
defects in, 437
fermentation characteristics of, 435-36
nutrition of, 436-37
taxonomy of, 434-35
Steroids, as possible growth factors, 64
Stickland reaction, 93
Streptomycin, 168-69
action of, 168
dependence upon, 168-69
effect of, on bacterial nucleus, 36
growth dependence upon, 187
resistance to, 168-69
and tuberculosis, 169
Streptomycin-dependency, factors influencing, 65
Substrates, for microbial food production, 460-63
Sucrose, oxidation of, 79
Sugars, differences in growth on and fermentation of, 84
Sulfate reduction, commercial aspects of, 109
Sulfur oxidation, 113
Sulphite liquor, utilization of, 461
Suppressor and modifier action, 197-98
Syncytial mass formation, viruses inducing, 490
Synergistic action, in bacterial nutrition, 53

T

Taxonomy
of chemoautotrophic bacteria, 106-7
of starter cultures, dairy, 434-35
Temperate phages, 247-51
Temperature
and bacterial nutrition, 47-48
and phage behavior, 248
and radiation damage, 220
and yeast growth and fermentation, 464
Temporary variations, 194-95
Terramycin (oxytetracyclene)
effect of, on bacterial nucleus, 36
see also Tetracyclenes
Tetracyclenes
action of, 170
preparation of, 170
properties of, 170
Tetrad analysis, and haploid genetics, 209-10
Thiosulfate oxidation, 112
Threonine, biosynthesis of, 95, 182
Thymine, sparing effect on pteroylglutamic acid, 51
Tissue cultures
cytopathology of virus infections in, 473-502
effect of viruses in, 403-4
factors influencing growth of viruses in, 475
Tissue extracts, and study of resistance, 374-78
Tissue metabolites, and bacterial growth, 377-78
Tobacco mosaic virus, as labelled antigen, 345-47
Toxemia, of infections, 386
Toxins, 424-26
associated with protein complexes, 426
bacterial, 411-28
definition of, 411-12
purity of, 424
Transadolase, 82
Transaminase, 95-96
Transaminase B, and amino acid synthesis, 182
Transaminases, types of, 93
Transamination, 58, 93
Transdehydrogenase, and phosphorylation, 75
Transduction, 198, 208
of glagellar determinants, 6
role of phage in, 251
Transformation, 208-9
Transglycosidation, 85
Transhydrogenase, 74-75
Transphorase, 90
Tricarboxylic acid cycle
in microorganisms, 80
in photosynthetic bacteria,

119
Triphenyltetrazolium chloride, as reduction site indicator, 10
Tryptophan, biosynthesis of, 59-60
Tubercle bacilli
action of streptomycin upon, 169
tissue extracts inhibiting, 376-77
Tuberculosis, and streptomycin, 169
Tumors
effect of viruses on, 393-410
and virus growth, 406-8
in man, effect of viruses on, 404-6
Tumor-producing viruses, cellular changes induced by, 496-97
Tyrosine
biosynthesis of, 59-60
formation from phenol, 91
metabolism of, 97

U

Ultraviolet light, reactivation by, 221
Urethane
factors influencing activity of, 83
nitrification of, 110-11
Uridine diphosphate acetylglucosamine in yeast, 84

V

Vaccinia virus
cell inclusions and degeneration, 488-89
effect of
on mouse leukemias, 401
on tumors in man, 405
Valine, biosynthesis of, 182
Variations
of influenza viruses, 317-18
mechanisms for, in influenza viruses, 315-22
persistent, 192-94
PQR, of influenza A, 314-15
temporary, 194-95
Varicella virus
cellular changes induced by, 491-92
effect of, on tumors in man, 405
Viral cytopathogenicity
definition of, 473-502
morphological manifestations of, 475-93
Viral growth, in tissue culture, factors influencing, 474
Viral hepatitis, 289-98
Viral recombination, mechanism for, 320-22
Virulence
and amino acid requirements, 183
and salt agglutination, 216
significance of capsules and surfaces in, 3
and transformation, 209
Virus
dengue, 301
of infectious hepatitis, 289-95
infections
cytopathology of, 473-502
of serum hepatitis, 296-97
Virus III
effect of, on tumors, 399-400
cell inclusions and degeneration, 489-90
Viruses
agents active against, 173
and cellular degeneration, 476-83
acute respiratory disease, 476
epidemic exanthem, 483
Coxsackie, 476-77
Equine encephalomyelitis, 477-78
Foot and mouth, 478-79
Newcastle, 479-80
Poliomyelitis, 480-82
undifferentiated fecal, 482-83
cytopathogenicity and, general comment, 497-98
effect of
on mouse ascites tumors, 401-3
in tissue cultures, 403-4
on tumors, 393-410
on tumors in man, 404-6
of ill-defined cytopathogenicity
fowl pox, 493
influenza, 493-94
mumps, 494
neoplastic, 496
rabies, 494-95
Rift Valley fever, 495
salivary gland disease, 495
St. Louis encephalitis, 495
yellow fever, 495-96
tumor-inducing, 496
inclusion body formation and cellular degeneration by, 483-90
adenoid degeneration, 484
ectromelia, 484
equine abortion, 485
fowl plague, 485-86
infectious canine hepatitis, 486
lymphogranuloma inguinale, 486-87
pseudorabies, 486
psittacosis, 487-88
vaccinia, 488
virus III, 489-90
influenza, antigenic variation of, 311-32
miscellaneous, effect of
on mouse leukemias, 401
in tissue culture, 404
multinucleate cell formation by
herpes simplex, 490-91
herpes zoster, 491-92
varicella, 491-92
plant, RNA in, 217
proteins of, 217-18
recombination in, 202-3
synctial mass formation by
measles, 492-93
rhesus monkey renal tissue, 493
theories for reproduction of, 321-22
and tumor growth, 406-8
see also specific viruses
Vitamin B_6, 55-56
antagonists for, 55-56
inhibition of isoniazid toxicity by, 56
Vitamin B_{12}
assay, influence of NaCl on, 51
in fungi, 458
oxidation products, toxicity of, 51
requirements of microorganisms, 49-51
requirement of bacteria, and O/R potential, 48
reversal of molybdenum toxicity by, 51
sparing effect on pteroylglutamic acid, 51
stimulation of acetate oxidation by, 50
toxicity of degradation products of, 50
Vitamins, biosynthesis of, 183-84

W

Wastes, utilization of, 460-63
Weil's disease, 141-42
West Nile virus
effect of
on mouse leukemias, 400
in tissue cultures, 404
on tumors in man, 405
Whey, utilization of, 460-61
Wood hydrolysates, utilization of, 461-62
Worm infections, polysaccharides as test antigens for, 355
Worms, immunological properties of polysac-

charides of, 353-62

X

Xylose, dissimilation of, 82
Xylulose, 82

Y

Yeast
genetics of, 210-12
mating types in, 210-11
respiration deficient mutants of, 81-82

Yellow fever, 298-300
animal reservoirs of, 299
epidemiology of, 299
vaccine against, 299-300
virus
cellular changes induced by, 495-96
effect of, on tumors, 400